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Instruction to Authors

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Journal

Joshi BK, S Gyawali and DS Poudyal. 2002. Regression analyses and multiple comparison procedures: Uses and misuses. J. Institute Sci. Tech. 12:69-81.

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tillers allowing penetration of sunlight and good aeration. The flag leaves are straight up-right with length of more than 20 cm above panicles. These morphological characters allow less contact among tillers, thus negate disease spread. This is probably the reason that *Sabitri* and *Tetep* were extensively used as donor parents in breeding program considering plant morphology as a model in selecting the genotypes with similar plant stature for improving ShB resistance in rice.

Table 3. Resistance in rice genotypes to sheath blight under field conditions in 2011 wet season

IRRI accession	Genotype	Disease incidence (%)			AUDPC [‡]
		14 DAI	21 DAI	28 DAI [†]	
IRGC-32576	TETEP	0	0	13.33 G	46.66 H
IEGC-24154	IR 26	0	0	16.13 FG	56.45 GH
IR86174-17-15-11-23	SHB-134-11-23	0	1.852	19.16 FG	80.04 F-H
MTU-1010	MTU-1010	1.852	1.515	20.69 FG	89.50 E-H
IRGC-39723	RNR 57979	0.952	5.119	16.07 FG	95.40 D-H
IR86174-17-5-18-45	SHB-129-18-45	1.667	4.227	17.72 FG	97.46 D-H
IRGC5565	SABITRI	1.389	4.568	17.56 FG	98.31 D-H
IRGC-39932	SI 51	2.279	3.262	21.98 E-G	107.8 C-H
IRGC-19907	IR 24	0	5.681	20.39 FG	111.1 C-H
IR86174-17-15-11-44	HSB-134-11-44	0	6.277	20.07 FG	114.2 C-H
IR86174-17-15-11-42	SHB-134-11-42	3.03	8.73	12.73 G	116.3 C-H
IRGC-77301		2.646	4.884	22.66 E-G	122.8 C-H
RTP11793	RNP74229	0.813	6.389	22.29 E-G	125.6 C-H
IRGC-38624	BR 3	2.464	7.898	21.48 E-G	139.1 C-H
IRGC-40020	LANKANDA 41	3.171	6.624	24.89 C-G	144.6 B-H
IRGC-47111	RAJESHWARI	0	5.808	33.09 B-G	156.5 B-H
IRGC-117278	SWARNA	1.235	10.345	23.72 D-G	159.7 B-H
IRGC-59244	TARA	1.235	11.765	28.37 B-G	186.0 B-H
IRGC-53200	MATAHAMBRE	3.889	13.041	40.52 B-G	246.7 B-H
IRGC-6264	N-22	1.389	16.186	41.86 B-G	264.7 B-G
IRGC77471	YUEFU	5.635	15.588	40.92 B-G	272.1 B-F
IRGC116438	AZ(C)	0	16.667	44.74 B-G	273.3 B-F
IRGC-117398	VANDANA	2.564	9.804	63.09 A-D	298.4 B-E
IRGC-4819	N-22	4.312	9.283	63.72 A-C	303.1 B-D
IRGC-74540	TOMOE MOCH	3.935	11.997	61.11 A-E	311.6 BC
IRGC-19379	N 22	0	16.987	56.02 A-F	315.0 BC
IRGC-62172	KAMMRA	0	4.762	90.20 A	349.0 AB
IRGC82775	IAC 165	6.278	36.84	67.42 AB	515.8 A
LSD _{0.05}		NS	NS	33.47	172.3

[†] Values followed by different letters are significantly different at (P = 0.05)

[‡] Values followed by different letters within the column are significantly different at (P = 0.05)

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Genetic Parameters of Common Wheat in Nepal

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ABSTRACT

Knowledge on variation within traits and their genetics are prerequisites in crop improvement program. Thus, in present paper we aimed to estimate genetic and environmental indices of common wheat genotypes. For the purpose, eight quantitative traits were measured from 30 wheat genotypes, which were in randomized complete block design with 3 replicates. Components of variance and covariance were estimated along with heritability, genetic gain, realized heritability, coheritability and correlated response. Differences between phenotypic and genotypic variances in heading days, maturity days and plant height were not large. Grain yield and plant height showed the highest phenotypic (18.189%) and genotypic (12.06%) coefficient of variances, respectively. Phenotypic covariance was higher than genotypic and environmental covariance in most of the traits. The highest heritability and realized heritability were of heading days followed by maturity days. Genetic gain for plant height was the highest. Co-heritability of 1000-grain weight with tillers number was the highest. The highest correlated response was expressed by grain yield with tillers number. This study indicates the possibility of improving wheat genotypes through selection utilizing existing variation in these traits.

Key words: Covariance components, genetic parameters, *Triticum aestivum*, variance components

सारांश

बालीको जातीय विकासको लागि वंशाणुगत गुणहरूको विविधता र यी गुणहरूको अनुवंश बारे अध्ययन गर्नु आवश्यक हुन्छ। वंशाणुगत गुणहरू बारे विभिन्न आठ वटा गुणहरूको ३० वटा गहुँको जातहरूमा आर.सी.वि.डी. प्रयोग गरि तीन छुट्टाछुट्टै सेटमा वंशाणुगत गुणहरूको मात्रा जस्तै genetic gain, heritability, coheritability, variance-covariance components आदिको अध्ययन गरिएको थियो। फूल फुल्ने अवधि, पाक्ने अवधि र बोटको उचाईमा phenotypic/genotypic विविधता बीच धेरै फरक नभएको पाइयो। सबै भन्दा बढी phenotypic coefficient of variance (१८.१८९%) गहुँको दानाको तौलमा र genotypic coefficient of variance (१२.०६%) बिरुवाको उचाईमा पाइयो। अधिकतर गुणहरूमा genotypic/environmental covariances भन्दा phenotypic covariance बढी भएको देखियो। फूल फुल्ने र पाक्ने अवधि जस्ता गुणहरूको heritability र realized heritability सबै भन्दा बढी पाइयो। सबै भन्दा बढी आनुवंशिक उपलब्धि बालीको उचाईमा थियो। हजार दानाको तौल, tiller संख्या संगको coheritability सबै भन्दा बढी थियो। बढी correlated response दानाको तौल र tiller संख्याले देखायो। यस अध्ययन बाट उक्त जातहरूमा आनुवंशिक विविधता भएको पाइएकोले उक्त जातहरू बाट नयाँ जात निकाल्न सकिने सम्भावना देखिन्छ।

INTRODUCTION

Varietal richness both in terms of landraces and modern variety including introduced and wild genotypes of wheat is high in Nepal (NARC 1997, Mudwari 1999, Upadhaya and Joshi 2003, Joshi et al 2004, Mudwari et al 2004, Joshi et al 2013). Altitudinal climatic variation and farmers' needs are probably the major attributers for high wheat diversity. Diversity study among quantitative traits and their genetic parameters estimates are prerequisite in wheat breeding program (Desheva and Kyosev 2015, Farshadfar and Estehghari 2014, Farshadfar et al 2013). Use of genetic resources requires their proper and systematic evaluation due to substantial variation of wheat varieties in India (Pathak and Nema 1985), Ethiopia (Belay et al 1993), Nepal (Joshi et al 2004), Iran (Farshadfar and Estehghari 2014), Bulgaria (Desheva and Kyosev 2015) and many other countries. Murty and Arunachalam (1966) stated that genetic drift and selection in different environments could cause greater diversity than geographical distance. Natural selection super-imposed by human under varied agro-climatic, soil and stress environmental conditions have produced marked variability that can be utilized for new varieties improvement and development (Murty and Arunachalam 1966). Currently, such characterization has been more crucial in the context of climate resilient varieties and resistant not only to diseases but also to drought (Desheva and Kyosev 2015, Farshadfar and Estehghari 2014). It should be recognized that improvement in one character as a result of selection for another depends not only on the genotypic and phenotypic correlations, but also on the genotypic and phenotypic variances associated with them (Johnson et al 1955).

Wheat is the third most important crop after rice and maize in Nepal. Different approaches have been employed to increase the productivity of wheat. Genetic improvement is the major technique to develop high yielding including drought tolerance and climate resilient varieties (Farshadfar et al 2013). Estimates of genetic parameters are important to efficiently breed the wheat. Information on genetic parameters is useful to plant breeders in order to design efficient breeding methods for crop improvement program. Considering these gaps, the objective of present study was to measure variance and covariance components, phenotypic and genotypic coefficients of variations, heritability (h^2), coheritability (ch^2), realized heritability (rh^2) correlated response (CRy) and genetic gain (ΔG), for selected important quantitative traits of wheat.

MATERIALS AND METHODS

Altogether 30 wheat genotypes (28 advanced lines and two-released cultivars) were evaluated in Initial Evaluation Trial at Agriculture Botany Division (ABD) in Khumaltar, Kathmandu valley in 2008. The ABD is located at 1350 m above the sea level. The climate of the experimental site is warm temperate mid hill agro-ecological condition. Some of these wheat genotypes were received from

International Maize and Wheat Improvement Center (CIMMYT), Mexico and some were developed by Agriculture Botany Division. Standard agronomical practices were followed using randomized complete block design with three replications. Eight traits namely heading days, maturity days, plant height, tillers number, grains number per spike, grains weight per spike, 1000-grain weight and grain yield were measured based on the descriptors (IBPGR 1985).

Based on these replicated data, variance, covariance components, and coefficients were estimated. Genetic parameters (heritability, genetic advance, genetic gain, selection differential, realized heritability, coheritability and correlated response) were computed. These were estimated following the procedures of Mudwari (1985), Johnson et al (1955), Robinson et al (1949), Burten and De Vane (1953) and Singh and Chaudhary (1985). Data were processed in MS Excel and analyzed in MINITAB and AGROBASE software.

RESULTS

The highest genetic coefficient of variation (GCV) was recorded for plant height (12.06%) followed by grain yield (9.92%), while lowest GCV was observed for headings and maturity days (Table 1). Phenotypic coefficient of variance (PCV) was usually higher than GCV, but the difference was very low (Table 1). The higher GCV and PCV for grain yield were (9.926% and 18.169%), plant height (12.06% and 12.96%) and tillers number (9.67% and 14.89%), respectively. The highest PCV was shown by grain yield (18.169%) followed by grain weight per spike (17.199%) (Table 1).

The heritability (h^2) estimate varied from 16% for grain weight/spike to 94% for heading days (Table 2). Moderate to high heritability estimates were found for 1000-grain weight, tillers number, and plant height. The estimate of h^2 for grain yield, grain weight/spike and grain number were per spike less. The genetic gain expressed as a percentage of the mean, ranged from 5.185 for maturity days to 23.104 for plant height. Heading days, maturity days, grain number/spike and grain weight per spike had relatively low values (Table 2).

To assess the tendency of various quantitative traits values for genotypic, phenotypic and environmental components, covariance and coheritability of 30 genotypes were determined (Table 3 and 4). Though most of the traits were inherited together, tiller numbers exhibited much higher values of coheritability with grain number per spike and 1000-grain weight. High coheritability was estimated amongst the traits especially grain yield reflected closer association and lesser environmental influence. The changes brought about through indirect selection ie correlated response (CRy) on an associated trait are given in Table 5. The highest CRy value was shown by grain yield with tillers number.

Table 1. Variance components of phenotype, genotype and environment and coefficient of variation with mean of 30 wheat genotypes (Symbols: Vp, Phenotypic variance. Vg, Genotypic variance. Ve, Environmental variance. PCV, Phenotypic coefficient of variation. GCV, Genotypic coefficient of variation)

Trait	Vp	Vg	Ve	PCV, %	GCV, %	Mean
Heading days	23.187	21.821	1.366	4.118	3.994	116.944
Maturity days	18.017	16.355	1.662	2.773	2.642	153.078
Plant height, cm	142.820	123.520	19.300	12.968	12.060	92.156
Tillers number	3017.333	1274.333	1743.000	14.891	9.677	368.878
Grain number/spike	32.087	5.297	26.790	15.754	6.401	35.956
Grain wt/spike, g	0.080	0.013	0.068	17.199	6.807	1.648
1000-grain wt, g	21.383	10.983	10.400	10.073	7.220	45.905
Grain yield, kg/ha	207297.333	61866.333	145431	18.169	9.926	2505.871

Table 2. Genetic parameters estimated from 30 wheat genptypes

Trait	Heritability (h^2)	Realized heritability (rh^2)	Expected genetic advance (GA)	Actual Genetic gain (ΔG)	Selection differential (S)
Heading days	0.941	0.941	9.335	7.983	8.482
Maturity days	0.908	0.907	7.937	5.185	5.712
Plant height	0.865	0.864	21.292	23.104	26.714
Tillers number	0.422	0.422	47.790	12.956	30.675
Grain number/spike	0.165	0.165	1.926	5.357	32.453
Grain wt/spike	0.157	0.156	0.091	5.549	35.430
1000-grain wt	0.514	0.513	4.893	10.659	20.751
Grain yield	0.298	0.298	279.914	11.170	37.428

Table 3. Phenotypic, genotypic and environmental covariance estimated from 30 wheat genotypes

Trait		2	3	4	5	6	7	Grain yield
Heading days	P _{cov}	16.060	-13.730	-26.325	0.907	0.165	0.768	-108.128
	G _{cov}	15.344	-13.559	-16.331	0.233	0.014	0.489	2.754
	E _{cov}	0.716	-0.171	-9.995	0.674	0.151	0.279	-110.882
Maturity days	P _{cov}		-18.891	-47.643	5.187	0.107	-0.445	144.139
	G _{cov}		-20.395	-37.831	5.458	0.124	0.313	191.899
	E _{cov}		1.504	-9.812	-0.270	-0.017	-0.759	-47.760
Plant height	P _{cov}			-71.626	-5.595	0.564	9.378	-280.233
	G _{cov}			-112.209	-8.617	0.383	8.745	-775.469
	E _{cov}			40.583	3.021	0.180	0.633	495.236
Tillers number	P _{cov}				-21.446	2.144	-4.199	13753.840
	G _{cov}				-61.458	-2.100	-27.371	5871.005
	E _{cov}				40.012	4.244	23.172	7882.835
Grain number/spike	P _{cov}					1.479	-4.625	273.031
	G _{cov}					-0.063	-3.368	33.298
	E _{cov}					1.542	-1.257	239.733
Grain wt/Spike	P _{cov}						0.485	47.818
	G _{cov}						0.258	14.646
	E _{cov}						0.227	33.172
1000-grain wt	P _{cov}							189.764
	G _{cov}							312.688
	E _{cov}							-122.924

P_{cov}, Phenotypic covariance. G_{cov}, Genotypic covariance. E_{cov}, Environmental covariance

Table 4. Coheritability (ch²) among quantitative traits in 30 wheat genotypes

Trait	Heading	Maturity	Plant	Tillers	Grain	Grain	1000-grain	Grain yield
Heading days		0.955	0.988	0.620	0.257	0.087	0.637	-0.025
Maturity days			1.080	0.794	1.052	1.159	-0.703	1.331
Plant height				1.567	1.540	0.680	0.932	2.767
Tillers number					2.866	-0.979	6.519	0.427
Grain						-0.043	0.728	0.122
Grain wt/spike							0.532	0.306
1000-grain wt								1.648

Table 5. Correlated response (CRy) among quantitative traits in wheat

Trait	Heading days	Maturity days	Plant height	Tillers number	Grain number/spike	Grain wt/spike	1000-grain wt	Grain yield
Heading days		6.564	-5.800	-6.986	0.099	0.006	0.209	1.178
Maturity days			-9.898	-18.360	2.649	0.060	0.152	93.131
Plant height				-6.022	-0.462	0.021	0.469	-41.616
Tillers number					-2.305	-0.079	-1.026	220.175
Grain number/spike						-0.023	-1.225	12.109
Grain wt/spike							1.875	106.456
1000-grain wt								139.297

DISCUSSION

The magnitude of heritable variability, more particularly its genetic component is the most important aspect of the genetic constitution of the breeding material, which has a close bearing on the response to selection. For this determination, it was necessary to divide the total phenotypic variance of the quantitative traits into its components as these are the basis for genetic analysis, because the dimensions of these components decide the breeding behavior of the population and provide the bases of selection techniques (Moghaddam et al 1998). The contribution of environmental factors was substantially important on total variance because of the lower values of GCV in comparison to PCV. Farshadfar and Estehghari (2014), Desheva and Kyosev (2015) also reported similar result. GCV gives a quantitative measurement of genetic variability in a particular trait. The highest genetic coefficient of variation in plant height followed by grain yield indicates the effective selection for these traits. Heading days (3.99%) and maturity days (2.64%) exhibited lower values of GCV suggesting that these traits are influenced by environment. Therefore, the selection for these traits will not be effective. Similar results were also obtained by Mudwari (1985). Since, most of these traits are controlled polygenically and inherited quantitatively, thus substantial environmental influence might impact on these traits. Maturity days, heading days, plant height and tillers number were exhibited higher genetic variance than environmental variance (Table 1) indicating thereby a lesser influence of environment upon these traits. These traits had also high heritability values (Table 2).

High genotypic variance for a particular trait may not reflect heritable proportion of variation. Estimates of variance and its components alone are not helpful in determining the heritable portions of variation (Falconer 1960). For this, an estimation of heritability (h^2), the heritable proportion of variances of the traits seems necessary. High genetic gain was observed for plant height, tillers number, 1000-grain weight and grain yield. Similar results were observed by Moghaddam et al (1998) confirming the validity estimates of present results.

In the present study, the estimate of h^2 was large for 1000-grain weight (0.514), plant height (0.865), heading days (0.941), low for grain yield (0.298) and grain number (0.165) (Table 2). These values were similar to the findings of Sikka and Jain (1958) showing the validity of the findings. Bhullar et al (1982) in genetic analysis of durum wheat reported low h^2 estimates for grain yield, spikes per plant, medium for spikelets per spike, grain per spike, 1000-grain weight and plant height. Heritability estimates reported for grain yield in wheat were usually low (Gandhi et al 1964, Pathak and Nema 1985, Mudwari 1985). High GCV value with intermediate estimates of h^2 and genetic gain in this study agreed with Belay et al (1993).

Since environmental variance is dependent upon the conditions of culture or management, more variable (fluctuating) conditions reduce h^2 , contrarily higher uniformity increases it. The values of h^2 computed in this study for wheat refer to the populations grown in Khumaltar, Kathmandu Valley or similar condition elsewhere to great extent. However, such values for other populations in other situations could be liable to be more or less similar according to the population and the environmental conditions. The genotype-environment interaction variance was not separated from the genetic variance in this study, therefore the estimates of GCV, h^2 and genetic gain might be inflated.

Genetic gain along with high h^2 is more useful in predicting the resultant effects of selection (Johnson et al 1955). Plant height exhibited the highest genetic gain, whereas tillers number, 1000-grain weight and grain yield showed substantially high response to selection (Table 2). Three characters i.e. plant height, tillers number and 1000-grain weight exhibiting high h^2 along with higher genetic gain suggests that these characters can also be improved by selection. The low genetic gain against high h^2 estimate for heading days and maturity days showed that the expression of these characters were dependent to non-heritable variability up to certain extent.

The expected genetic advance (GA) was the highest on grain yield, but the highest genetic gain was observed on plant height (Table 2). The gain on grain yield from selection expressed as a percentage of the mean was 11.17%. This means that if the top 5% of the wheat plants were chosen, the grain yield would be expected to increase by 11.17% after one cycle of selection.

Grain yield, 1000-grain weight, tillers number and plant height possessed substantially higher genetic variability and also exhibited higher genetic gains revealing greater response to the selection. These observations agreed with the results reported by Belay et al (1993) and Moghaddam et al (1998). A low GCV and low GA were observed for heading days and maturity days (Table 1 and 2). These indicated that the characters were under high environmental influence thus, selection based on these characters would be ineffective. The high value of GCV, h^2 and GA estimated for plant height, tillers number and 1000-grain weight indicated the predominance of additive gene action suggesting direct phenotypic selection based on these traits would be effective for varietal improvement.

The h^2 and rh^2 values were similar in four traits and rh^2 was slightly lower than h^2 in others four traits (Table 2). The rh^2 is primarily a description of the response and may not provide a valid estimate of the h^2 in the base population (Falconer, 1960). Larger population tends to give response to selection due to mainly to larger realized h^2 . Days to maturity and 1000-grain weight have played important role to increase grain yield because of high genetic covariance of these traits with grain yield (Table 3). Selection based on the number of tillers and grain number per spike could not be considered effective to increase yield due to high environmental covariance. High genotypic covariance between grain yield and weight was also reported by Farshadfar and Estehghari (2014). High values of genotypic covariance are very useful in breeding program to improve the traits simultaneously. Coheritability estimates were positive for most of traits (Table 4). The high coheritability values of grain yield with maturity days, plant height and 1000-grain weight indicate that selection on these traits would affect largely on grain yield. Farshadfar and Estehghari (2014) observed high coheritability values of grain yield with other agro-morphological traits.

If the trait under selection is genetically correlated with other trait(s), one can expect improvement in the correlated trait(s) as well. Thus, improvement in a trait can be made by direct as well as indirect selection. If we consider a plant height as selection criteria, negative indirect result will be observed in grain yield (Table 5). Because of the linkage relationship, correlated response frequently occurs under selection.

Knowledge of the genetic parameters of a population is useful in designing an effective breeding program. High h^2 and GCV (and therefore high genetic advance) are positive indication that the variation in these traits is attributable to a high degree of additive genetic effect. Selection for these characters should be effective and satisfactory for practical purpose. Information on these aspects will be valued by wheat breeders.

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Soil Physico-Bio-Chemical Properties under Poplar + Indian Mustard Inter Cropping System

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ABSTRACT

A field experiment was conducted during the winter seasons of 2008-10 at Agroforestry Research Centre, Pantnagar, India with aim to examine the effect of different levels of recommended Nitrogen (N): Phosphorus (P): Potassium (K) (NPK) on soil physico-bio-chemical properties under varying poplar tree densities with mustard intercropping. Lower soil bulk density was recorded under 1000 trees/ha density compared to sole crop in both the year. Soil bulk density (BD) decreased underneath trees. However, soil pH, available N and K were not influenced by tree density in both the years. Electrical conductivity (EC) and soil organic carbon (SOC) increased underneath trees of either tree density compared to sole crop in both the years. Significantly ($P < 0.05$) higher available P was recorded under 1000 trees/ha density compared to 250 and 500 trees/ha densities including sole crop in 2008-09. Similarly, soil microbial biomass carbon (SMBC) increased with increasing the tree density and significantly ($P < 0.01$) higher value was recorded under 1000 trees/ha density compared to sole crop and sparse density both the years except 2009-10, where 500 and 1000 trees/ha densities remain statistically at par. On the other hand, dehydrogenase activity (DA) was maximum under 500 trees/ha density compared to sole crop and 1000 trees/ha density in both the years. Among the fertility levels, the maximum SOC and available N were recorded with 75% compared to 50, 100 and 125% of recommended NPK in 2008-09, except available N with 100% of recommended NPK. But, available P was more with 100% of recommended NPK in 2008-09. Similarly, maximum SMBC were recorded with 75 % compared to higher doses of recommended NPK in both the years. Similarly, BD, EC, SOC, available N, P and K were recorded maximum and soil pH minimum in 0-15 cm soil layers compared deeper layers.

Key words: Fertility levels, pH, available N, P and K, DA

सारांश

माटोको भौतिक, जैविक तथा रसायनिक गुणमा विभिन्न सिफारिश मात्राको नाइट्रोजन, फास्फोरस तथा पोट्यासको लहरे पीपलका विभिन्न घनत्व र खुला स्थानमा परीक्षण गर्ने उद्देश्यले सन् २००८ देखि २०१० को हिउँदे सिजनमा तेलवाली लगाई गोविन्दबल्लभ पंत कृषि तथा प्रौद्योगिकी विश्वविद्यालय, भारतको कृषि वन अनुसन्धान केन्द्र, पथर चट्टामा परीक्षण संचालन गरिएको थियो। माटोको खडिलोपन (स्वायल बल्क डेन्सिटी) लहरे पीपलका रुखहरू मुनि घटेको र १००० रुख प्रति हेक्टर लगाइएको खेतमा रुख नभएको खाली खेत (एकलवाली) को तुलनामा तात्त्विक रूपमा दुवै वर्ष कम मापन गरिएको थियो। यद्यपि माटोको अम्लियपन, उपलब्ध नाइट्रोजन तथा पोट्यासियमको मात्रा दुवै वर्षमा विभिन्न घनत्वका रुखले प्रभावित पारेको पाइएन। विधुतिय चालकता (इलेक्ट्रिकल कन्डक्टिभिटी) तथा माटोको जैविक कार्बन दुवैवर्ष सबै घनत्वका रुखहरू मुनि खाली खेतको तुलनामा बढेको पाइयो। सन् २००८ र ०९ मा माटोको उपलब्ध फास्फोरसको मात्रा १००० रुख प्रति हेक्टरको घनत्वमा २५० र ५०० रुख प्रति हेक्टर र खाली खेत भन्दा तात्त्विकरूपले बढी मापन गरियो। त्यसैगरी, माटोको सुक्ष्म जिवाणुहरूको जैविककार्बन (स्वायल माइक्रोबियल बायोमास कार्बन) लहरे पीपल रुखका घनत्वहरू बढदै जाँदा बढेको पाइयो र खाली खेत र पातलो घनत्व भएका रुखहरूको खेतमा भन्दा १००० रुख प्रति हेक्टर भएको खेतमा बढी मापन गरियो। अर्को तर्फ दुवै वर्ष डिहाइड्रोजेन एक्टिभिटी (इनजाइमेटिक गतिविधि) ५०० रुख प्रति हेक्टर घनत्व भएको खेतमा, खाली खेत र १००० रुख प्रति हेक्टर भएको खेतमा भन्दा बढी रहेको पाइयो। २००८ र ०९ मा सबभन्दा बढी माटोको प्राज्ञारिक कार्बन र उपलब्ध नाइट्रोजनको मात्रा ५०, १०० र १२५५ भन्दा ७५५ सिफारिश मात्राको नाइट्रोजन, फास्फोरस, पोट्यासियम प्रयोग गरेको खेतमा पाइयो। तर, २००८ र ०९ मा उपलब्ध फास्फोरस १००५ सिफारिश मात्राको नाइट्रोजन, फास्फोरस, पोट्यासियम प्रति हेक्टर दिएको खेतमा बढी मापन गरियो। त्यसै गरी, माटोको सुक्ष्म जैविककार्बन पदार्थ उच्च मात्रामा भन्दा ७५५ सिफारिश मात्राको नाइट्रोजन, फास्फोरस, पोट्यासियम प्रति हेक्टर दिएको खेतमा दुवै वर्ष बढी मापन गरियो। माटोको घनत्व, विधुतिय चालकता, माटोको जैविक कार्बन, उपलब्ध नाइट्रोजन, फास्फोरस, पोट्यासियम जस्ता परिवर्त्यहरू (Variables) माथिल्लो सतहको माटो (०-१५ से.मी.) मा तल्लो सतहको माटोमा भन्दा बढी मापन गरियो भने माटोको अम्लियपन माथिल्लो सतहमा कम रहेको पाइयो।

INTRODUCTION

Soil fertility deterioration, in general, and organic matter depletion in particular under intensive agriculture system are the main problems in the tropical and sub-tropical region of Indian sub-continent. To certain extent agro-forestry land use system could be an alternate to reduce land degradation. Recently, Chauhan et al (2010) suggested Poplar (*Populus deltoides* Bartr) based agro-forestry as one of the viable land use options to prevent land degradation by which biological production could be restored sustainably. This system has been quite popular in northern India with expanding coverage every year. In such a system organic carbon content in soil (0-15 cm) has been shown to be increased by 35.6 % after 6 years of poplar planting than in land having single wheat crop (Chauhan et al 2010). As such, substantial loss in wheat yield occurred under Poplar, but compensated by the trees in terms of biomass, economics and carbon mitigation potential. There is need to increase the level of soil organic carbon not only to maintain land productivity, but also to provide a sink for atmosphere CO₂ in the terrestrial carbon pool. Moreover, the deposition of Poplar leaf litter changes C:N (Carbon:Nitrogen) ratio of the soil compensating potential crop growth.

The leaf litter biomass may vary depending on the tree age, density and management, hence nutrient cycling may also vary because soil biological activities are influenced by trees canopy, thereby availability of nutrients to underneath crops (Pingale 2009). The graded levels of fertilizers applied in inter cropping system invariably used by both crop as well as trees in agri-silvicultural system (Prasadini and Sreemannarayana 2007). However, such knowledge has rarely been examined in specific agri-silviculture system. Therefore, the present paper attempt to provide empirical knowledge on residual effect in soil in terms of physical, chemical and biological properties having varying Poplar tree density underneath Indian mustard with various levels of fertilizers applied.

MATERIALS AND METHODS

The field experiments were conducted at Agroforestry Research Centre Patharchatta of Govind Bhallav Pant University of Agriculture and Technology, Pantnagar, Uttarakhand located at 29°N Latitude, 79.3°E Longitude and at an altitude of 243.84 m above mean sea level during 2008-09 and 2009-10. The Poplar clone G4 were planted during February 2000 and mortality was maintained by gap-filling in following year to maintain 16 trees in each density at five meter row distance. The soil of the experiment site was clay loam (0-15 cm) in texture, having pH 8.0-8.05, organic carbon 0.99-1.17%, available nitrogen (N) 143.3-141.4 kg/ha, available phosphorus (P) 11.7-12.7 kg/ha and available potassium (K) 133.9-136.7 kg/ha in 0 to 45 cm soil layer under treeless (sole crop) to 1000/ha tree density plots. The land was thoroughly ploughed and incorporated leaf litter into the soil two weeks before sowing. Upland rice and wheat cropping sequence were followed till the beginning of the present experiment. The field remained fallow between the two experiments in *khari* seasons of 2008-09 and 2009/10. The experiment was laid out in split-plot design taking four tree densities, i.e. Sole crop (open), 250, 500 and 1000 trees/ha plots with four fertility viz. 50%, 75%, 100% and 125% of recommended dose of fertilizer (RDF) for Indian mustard (*Brassica juncea* L.) as sub-plot treatment with four replications. The recommended fertilizer dose was 120 kg N, 40 kg P₂O₅ and 20 kg K₂O/ha. The NPK mixture (12:32:16) and urea (46% N) were used as a source of fertilizers in both the years. The doses of 50% N and 100% P and K for Indian mustard were applied into the soil at the time of sowing.

Indian mustard var. Kranti was inter-cropped on 8th Nov in 2008 and 7th Nov in 2009. The mustard seed was sown in lines 30 cm apart continuously keeping seed rate @ 5 kg/ha manually. The crop was irrigated only once with tube well water in both years at about 45 days after sowing (DAS). The remaining 50% N was top dressed after one week of irrigation. The crop was harvested on March 23rd and 26th, in 2009 and 2010, respectively when more than 80% pod turned yellow. Soil samples from each main plot in 0-15, 15-30 and 30-45 cm depths were collected before sowing and after harvest of mustard crop in both the years for assessing chemical properties viz. pH, Electrical Conductivity (EC), Soil Organic Carbon and available N, P and K.

-For soil microbial study, samples from each subplot at 0-15 cm soil depth were collected at peak flowering stage and stored in deep freeze for the determination of soil microbial biomass carbon and dehydrogenase activity. Bulk density was determined by Core sampler from each main plot at 0-15, 15-30 and 30-45 cm soil depths before sowing mustard crop. Soil samples were analyzed for pH (Jackson 1973), EC, SOC (Black 1965), available N (Subbiah and Asija 1956), P (Olsen et al 1954) and K (Jackson 1973) using standard procedures. The soil values of pH, EC, SOC, available N, P and K were determined depth wise in 0-15, 15-30 and 30-45 cm depths. Chloroform fumigation extraction (Jenkinson and Powlson 1976) and TTC assay method (Tabatabai 1994) were employed for the determination of soil microbial biomass carbon (SMBC) and dehydrogenase activity (DA), respectively.

RESULTS

Soil Physical Properties

Soil Bulk Density

The soil bulk density (BD) (g/cc) was influenced by tree density and soil depths in both the years, respectively. The soil bulk density decreased with increasing tree density (Table 1). The highest 1.498 g/cc soil bulk density was recorded in sole cropping (open plot) as compared to underneath trees density in 2008-09 and 2009-10, respectively. However, the differences in soil bulk density between 250 and 500, and 500 and 1000 trees/ha plots in first year; and amongst 250, 500 and 1000 trees/ha densities in the second year were insignificant. The soil bulk density increased with increase in the soil depth. However, 15-30 cm soil depth had significantly ($P < 0.05$) higher bulk density than 0-15 cm in 2009-10 (Table 1).

Soil Chemical Properties

Soil pH

The soil pH after harvest of mustard crop was not influenced by tree density and fertility levels both during 2008-09 and 2009-10, while the soil pH varied with soil depths in both the years (Table 2). The soil pH increased with increasing the soil depth up to 30 cm and declined in further deeper (30-45 cm). The higher (8.04 and 8.47) and lower (7.80 and 8.23) soil pH was observed in 15-30 and 0-15 cm soil depths, respectively in both the years.

EC of Soil

The EC (dS/m) of extract from saturated soil after harvest of mustard crop was influenced by tree density, fertility levels and soil depths in both the years (Table 2). The EC of soil increased with increased tree density. The maximum (0.610 and 0.508 dS/m) substantially higher EC was recorded under 500 trees/ha density in 2008-09 and under 1000 trees/ha density in 2009-10, respectively, as compared to sole crop (open plot) and 250 trees/ha density in 2008-09. EC was similar in open plot and sparse density (250 trees/ha) in first year and higher density in second year. Moreover, EC of soil underneath poplar trees was more compare to sole crop (open plot) in both the years. Among the fertility levels, the maximum (0.678 and 0.480 dS/m) EC was recorded with 50% compared to 75, 100 and 125% of recommended NPK in first year and with 100% compared to 75 and 125% of recommended NPK in second year, respectively. Whereas, the differences between 50 and 100% and 75 and 125% of recommended NPK in 2009-10 were not significant. The EC of soil decreased with increasing soil depth and the maximum (0.588dS/m) EC was recorded in 0-15 cm depth comparing to 15-30 and 30-45 cm layers in 2008-09 and 2009-10. However, the EC in 15-30 and 30-45 cm soil depths were almost similar in 2009-10.

Table 1. Soil bulk density as influenced by poplar tree density and varying soil depths

Treatment		Bulk density (g/cc)
A. Tree density (trees/ha)	2008-09	2009-10
Sole crop	1.402	1.498
250	1.328	1.389
500	1.313	1.389
1000	1.295	1.375
SEm ±	0.009	0.024
CD, 5%	0.030	0.078
B. Soil depth (cm)		
0-15	1.290	1.293
15-30	1.366	1.414
30-45	1.349	1.531
SEm ±	0.008	0.023
CD, 5%	0.024	0.069

Organic Carbon (OC) Content in Soil

The organic carbon (%) of soil after harvest of mustard crop was influenced by tree density, fertility levels and soil depths in both the years, except by fertility levels in 2009-10 (Table 2). The SOC (%) increased with increasing tree densities having maximum values 1.11 and 1.01% in 2008-09 and 2009-10, respectively. The highest OC was recorded under highest density (1000 trees/ha) in both years, compared to sole crop field. The differences between sole crop and 250 trees/ha and amongst 250, 500 and 1000 trees/ha densities in first year and 250, 500 and 1000 trees/ha densities in second year were somewhat closer. The SOC increased by 11 and 25%; 15 and 26% and 19 and 26% with each successive increase in tree densities from zero to 1000/ha in 2008-09 and 2009-10, respectively.

Among the fertility levels, the maximum (1.11%) soil OC was recorded with 75% of recommended NPK compared to 50, 100 and 125 % in 2008-09. The differences between 50, 100 and 125% of recommended NPK were not substantial with respect to SOC content in soil. Organic carbon of soil decreased with increasing soil depth having maximum (1.41%) recorded in 0-15 cm depth comparing to 15-30 and 30-45 cm depths in both years. However, 15-30 cm depth had higher SOC than 30-45 cm in both the years. Surface soil layer (0-15 cm) showed approximately two times (107 and 133%) more SOC. Whereas, the middle soil layer (15-30 cm) contained 53 and 45% more SOC than the lower most studied layer i.e. 30-45 cm.

Available Nitrogen in Soil

The available N in soil after harvest of mustard crop was not influenced by tree density in both the years, while fertility levels showed substantial effect only in 2008-09 and by soil depths in both the years (Table 3). The maximum (178.1 kg/ha) more soil available N was recorded with 75% as compared to 50 and 125% of recommended NPK in 2008-09. The differences between 50 and 100; 50 and 125 and 75 with 100% of recommended NPK were not substantial. The available N in soil decreased with increasing soil depths having the maximum (197.3 kg/ha) high soil available N at 0-15 cm depth in both years, comparing to 15-30 and 30-45 cm depths. At 15-30 cm depth higher available N compared to 30-45 cm depth occurred in both years. The maximum available N (kg/ha) was found in surface (0-15 cm) layer during both the years. The lowest available N was recorded in the lower most soil of 30-45 cm.

Available Phosphorus in Soil

The available P in the soil was significantly influenced both by tree density ($P<0.05$) and fertility levels ($P<0.01$) only in 2008-09, but by soil depths in both the years (Table 3). The maximum P (19.5 kg/ha) was occurred under 1000 trees per ha density compared to other densities including sole crop plots in year 2008-09. The differences amongst sole crop plots and under 250 and 500 trees/ha densities remained statistically non-significant ($P<0.05$). The available P in soil increased with increasing fertility levels up to 100% of recommended NPK, while the difference between 75 and 125% of recommended NPK was not significant.

Available Potassium (K) in Soil

The available K in soil after harvest of mustard crop was not influenced by tree density and fertility levels in any of the growing season, but varied with the soil depths in both the years (Table 3). Alike available N and P, available K in soil also decreased with increased soil depths. The maximum (200.9 and 224.5 kg/ha) available K in soil was recorded in 0-15 cm depth which decreased significantly ($P<0.05$) with soil depth both years due to more turn-over of organic residues on the surface soil. The lowest available K was recorded in the lower most soil layer of 30-45 cm.

Soil Biological Properties

Soil Microbial Biomass Carbon

Soil microbial biomass carbon (SMBC) was significantly ($P<0.05$) influenced by tree density and fertility levels in both the years (Table 4). SMBC increased with high tree densities having maximum (239.0 $\mu\text{g g}^{-1}$ soil), higher SMBC under highest (i.e. 1000 trees/ha) density both in 2008-09 and 2009-10, respectively, comparing other densities including sole crop in first year and sole crop and 250 trees/ha density in second year. The differences between 250 and 500 trees/ha and 500 and 1000 trees/ha densities were not significant ($P<0.05$) in 2009-10. Among the fertility levels, the maximum 227.88 $\mu\text{g g}^{-1}$ soil was significantly ($P<0.05$) higher in 75% of

recommended NPK, respectively, compared to 100 and 125% of recommended NPK both in 2008-09 and 2009-10. The differences between 50 and 75% and 100 and 125 % of recommended NPK were not significant ($P < 0.05$) in both the years.

Table 2. pH, EC and OC (%) of soil as influenced by poplar tree density, fertility levels and soil depths after harvest of mustard crop during both the growing seasons

Treatment	pH		EC (dS/m)		SOC (%)	
	2008-09	2009-10	2008-09	2009-10	2008-09	2009-10
A. Tree density (trees/ha)						
Sole crop	7.98	8.40	0.425	0.322	0.93	0.80
250	7.90	8.33	0.456	0.503	1.03	1.00
500	7.96	8.41	0.610	0.500	1.07	1.01
1000	7.93	8.36	0.587	0.508	1.11	1.01
SEm \pm	0.03	0.03	0.014	0.011	0.03	0.04
CD, 5%	NS	NS	0.051	0.040	0.12	0.16
B. Fertility levels (% of recommended NPK)						
50	7.93	8.36	0.678	0.466	1.02	0.96
75	7.93	8.38	0.431	0.452	1.11	0.91
100	7.96	8.39	0.468	0.480	1.00	0.98
125	7.96	8.37	0.500	0.434	1.01	0.97
SEm \pm	0.02	0.02	0.010	0.009	0.01	0.02
CD, 5%	NS	NS	0.030	0.026	0.04	NS
C. Soil depth (cm)						
0-15	7.80	8.23	0.588	0.487	1.41	1.40
15-30	8.04	8.47	0.530	0.441	1.04	0.87
30-45	7.98	8.42	0.441	0.447	0.68	0.60
SEm \pm	0.01	0.01	0.005	0.005	0.005	0.01
CD, 5%	0.03	0.04	0.014	0.015	0.01	0.03

NS-Not significant

Table 3. Available N, P and K in soil (kg/ha) as influenced by various poplar tree densities, fertility levels and soil depths after harvest of mustard crop during both the growing seasons

Treatments	Nitrogen		Phosphorus		Potassium	
	2008-09	2009-10	2008-09	2009-10	2008-09	2009-10
A. Tree density(trees/ha)						
Sole crop	175.5	159.7	14.5	19.0	131.8	145.0
250	175.2	169.5	15.3	19.0	158.3	158.3
500	155.1	162.4	14.7	18.7	129.2	159.0
1000	155.9	163.8	19.5	18.8	143.2	151.7
SEm \pm	7.9	3.2	1.0	0.6	12.6	16.6
CD, 5%	NS	NS	3.4	NS	NS	NS
B. Fertility levels(% of recommended NPK)						
50	158.4	160.9	13.2	18.0	140.5	150.4
75	178.1	164.3	15.8	19.6	142.5	147.0
100	173.6	164.3	18.4	19.7	138.7	159.4
125	151.5	165.9	16.6	18.3	140.8	157.2
SEm \pm	5.7	1.8	0.5	0.6	4.3	6.3
CD, 5%	16.6	NS	1.5	NS	NS	NS
C. Soil depth (cm)						
0-15	197.3	191.8	26.9	27.2	200.9	224.5
15-30	161.7	161.9	12.8	15.7	117.1	143.1
30-45	137.2	137.9	8.3	13.8	103.9	92.9
SEm \pm	3.1	1.3	0.3	0.5	3.1	3.6
CD, 5%	8.9	3.7	1.0	1.6	8.6	10.1

NS- Not significant

Dehydrogenase Activity

Dehydrogenase activity was significantly ($P < 0.05$) influenced by tree density during both the years. The dehydrogenase activity was not influenced by fertility levels during both the years (Table 4). Dehydrogenase activity increased with increased tree densities and reached maximum (118 and 167 $\mu\text{g TPFg}^{-1} 24 \text{ hr}^{-1}$) and significantly ($P < 0.05$) higher under 500 trees/ha density in 2008-09 and 2009-10, respectively, compared to sole crop in first year and sole crop, 250 and 1000 trees/ha densities in second year. The differences between sole crop and 1000 trees/ha and amongst 250, 500 and 1000 trees/ha in 2008-09 and between 250 and 1000 trees/ha densities in 2009-10 were not significant ($P < 0.05$).

DISCUSSION

The soil bulk density (BD) (g/cc) was substantially influenced by tree density and soil depths in both the years i.e. 2008-09 and 2009-10 (Table 1). The lower bulk density was recorded in higher density due to high organic matter content in closely spaced tree density. More leaf litter fall might occurred in plots under higher tree densities. Higher value of bulk density was observed in second year due to removal of leaf litter in first year from the mustard plot for establishment and proper growth of experimental crop (mustard), hence complete addition of litter to soil could not be allowed. These results are in close agreement with Nayak et al (2009) and Pingale, (2009). Nayak et al (2009) observed the lower bulk density under *Prosopis juliflora* tree as compared to open field. Pingale (2009) also observed decrease in soil bulk density with increased in *Populus deltoides* tree density. The soil bulk density increased with the increase in the soil depth due to poor organic matter content at deeper depth. Gupta and Sharma (2008) also reported soil physical properties such as porosity, density and water holding capacity improved in poplar plantation compared to sole crop in Uttarakhand.

The soil pH varied with soil depths in both the years (Table 2). The marginally lower pH in 0-15 cm soil depth under agroforestry systems may be due to substantial addition of organic matter to the surface soil under trees and organic acid released during litter decomposition as also reported in agrisilvicultural system by Prasadini and Sreemannarayana (2007) and Kumar et al (2008). Newaj et al (2007) also observed very nominal changes in soil pH under White siris (*Albizia procera*) based agrisilvicultural system after 4 years of experimentation as compared to initial value due to very high free calcium carbonate content in the soils. Bharadwaj et al (2001) observed increasing trend of soil pH with deeper soil layers under various tree densities of poplar.

EC of soil underneath poplar trees was higher compared to sole crop (open) in both the years (Table 2). Newaj et al (2007) also observed higher EC under *Albizia procera* based agrisilvicultural system than under pure crop of mustard and black gram. This could be due to enrichment of soil mineral basic salts through addition and decomposition of litter. Singh et al (2007) reported that poplar trees returns maximum amount of Ca into the soil through litter fall amongst different macronutrients.

Among the fertility levels, lower (50%) of NPK fertilized plots showed higher value of EC. Maji and Mandal (2004) also observed that addition of 75% of recommended NPK plus FYM either in both the seasons and single season enhanced the electrical conductivity of soil than the control in paddy-oat/berseem rotations. The EC of soil decreased with increasing soil depth. Higher magnitude of EC in 0-15 cm depth compared to lower depth occurred probably due to the presence of more litter at the surface soil. Similar results have also been shown by Newaj et al (2007) in agrisilviculture system in semi-arid areas of India.

Soil organic carbon contents decreased in 2009-10 compared to 2008-09, this might be due to defoliation of poplar leaves by insect (caterpillar) attack during early autumn season in 2009-10. It is speculated that the defoliation must have reduced the total biomass of organic matter addition into the soil in 2009-10. Such facts might need to be examined in future studies how the caterpillars might impact the biomass loss on soil organic carbon deposits. The SOC (%) in soil increased with increasing the tree densities compared to sole crop i.e. open field. The SOC (%) increased by 11 and 25%; 15 and 26% and 19 and 26% with each successive increase in tree density from zero to 1000/ha in 2008-09 and 2009-10, respectively. Previous studies have also argued that continuous litter addition and tree shading should helped in protecting soil organic matter by reducing oxidation (Gill and Burman 2002, Bharadwaj et al 2001, Pingale 2009). Swami and Puri (2005) also observed 15.1% increase over initial value in SOC (%) in *Gmelina arborea* based agrisilvicultural system, while it has been 51.2% increase in pure plantation of *G. arborea*.

Among the fertility levels, the maximum soil OC was recorded with 75% of recommended NPK compared to other levels of NPK. Das et al (2010) also observed highest soil organic carbon content with lower recommended NPK (50% NPK) in conjunction with *Azolla* compost compared to recommended NPK levels. Moscatelli et al (2008) observed fertilization did not modify soil capacity to accumulate organic matter in the medium term. Organic carbon of soil decreased with increasing soil depth and the maximum SOC was recorded in upper layer compared to deeper layers. Surface soil layer (0-15 cm) showed approximately two times (107 and 133%) more SOC (%) during 2008-09 and 2009-10, respectively. Whereas, the middle soil layer (15-30 cm) contained 53 and 45% more SOC (%) than the lower most layer i.e. 30-45 cm during 2008-09 and 2009-10, respectively. Similar variations in SOC with soil depth have also been indicated by Pingale (2009), Swami and Puri (2005) and Chauhan et al (2010).

The maximum soil available N was recorded with 75% compared to lower (50%) and higher levels (125%) of NPK. This may be due to depressive effect of higher dose of P on nitrogen availability in the soil. Haris et al (1999) had observed the depressed available N status of soils by application of P. The available N, P and K in soil decreased with increased soil depths and the maximum available N, P and K (kg/ha) was found in surface soil (0-15 cm) layer during both the years due to more turn-over of organic residues on the surface soil. The lowest available N, P and K was recorded in the lower most soil layer of 30-45 cm. Bharadwaj et al (2001) also observed decreasing trend in available N, P and K content with increase in soil depth even under high density poplar plantation.

Similarly, Swami et al (2008) also observed available nutrient (NPK) decreased with soil depth under *Gmelina arborea* based agrisilvicultural system. Maximum available P was recorded under highest (1000/ha) tree density probably due to the high organic matter content in the soils. The decomposition of organic matter is accompanied by the release of appreciable quantities of carbon dioxide which forms carbonic acid, when dissolved in water can decompose certain primary minerals. It has been shown that in calcareous soils, carbon dioxide production plays an important role in increasing the phosphate availability. The organic material forms a cover on sesquioxide and reduces the phosphate fixing capacity of the soil (Das et al 2010). The maximum available P in soil was obtained with 100% NPK probably due to the P content of soil increased with increased dose of NPK.

Table 4. Soil microbial biomass carbon ($\mu\text{g g}^{-1}$) and dehydrogenase activity ($\mu\text{g TPF g}^{-1} 24\text{h}^{-1}$) as influenced by poplar tree density and fertility levels at flowering stage during both the growing seasons

Treatments	Soil microbial biomass carbon		Dehydrogenase activity	
	2008-09	2009-10	2008-09	2009-10
A. Tree density(trees/ha)				
Sole crop	159.00	187.00	103.00	127.00
250	179.00	207.00	117.00	147.00
500	205.00	224.00	118.00	167.00
1000	226.00	239.00	109.00	145.00
SEm \pm	6.04	6.01	3.49	5.09
CD, 5%	19.31	19.22	11.15	16.28
B. Fertility levels (% of recommended NPK)				
50	197.73	219.70	113.03	141.80
75	203.10	227.88	112.98	150.70
100	185.41	205.29	111.39	153.40
125	180.82	203.35	109.79	140.60
SEm \pm	3.40	3.43	1.12	4.09
CD, 5%	9.75	9.83	NS	NS

NS=Not significant

Soil microbial biomass carbon increased with increased tree densities owing to higher organic carbon content, and favorable microclimate under higher density. Kumar et al (2008) also observed higher microbial activity under plantations of different tree species in the upper surface of soil due to presence of higher organic carbon content. Chander et al (1995) observed improved microbial biomass carbon during decomposition of poplar leaf litter compared to *Eucalyptus* leaf litter.

Higher SMBC was obtained with 75% of recommended NPK due to adverse effect of higher dose of chemical fertilizers on active microbial population growth. This finding is similar to Cheema et al (2008) where enzymatic activity in soils was found to be depressed with continuous application of recommended inorganic fertilizers. Dehydrogenase activity increased with increased tree densities and reached maximum under 500 trees/ha density. This was probably due to higher fine root biomass under 500/ha tree density. Since the roots are known to produce exudates which are likely to provide energy for microbial metabolic activity. Singh and Singh (1994) also observed fine roots biomass of poplar was more in wider spacing, generally in 0-15 cm soil layers. The dehydrogenase activity improved underneath trees comparing to sole crop in both the years indicating increased active microbial population, as it provides correlative information on the biological activity and microbial population in the soil. This improvement in dehydrogenase activity might be due to improved physico-chemical properties of soil by trees. Batra (2004) also observed the dehydrogenase activity of soil being influenced both by the soil physico-chemical characteristics and agricultural management practices.

Agroforestry land use system is viable for sustainable agricultural production due to substantial addition of organic matter into the soil. The soil quality in enhanced underneath trees and optimum tree density is required for proper crop growth under tree canopy. Optimum dose of NPK (75%) is required for maintaining the soil quality in agrisilviculture system.

Agriculture land is believed to be a major potential sink and could absorb large quantities of carbon, if trees are reintroduced to these systems and judiciously managed together with the crop and/or animals. Thus importance of agroforestry as a land use system is receiving a wider recognition not only in terms of agricultural sustainability, but also due to climate change. Litter fall plays an important role in terms of nutrient cycling, and thus adds to sustainability of the system.

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Wheat Yield Trend and Soil Fertility Status in Long Term Rice-Rice-Wheat Cropping System

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ABSTRACT

A long-term soil fertility experiment under rice-rice-wheat system was performed to evaluate the long term effects of inorganic fertilizer and manure applications on soil properties and grain yield of wheat. The experiment began since 1978 was laid out in randomized complete block design with 9 treatments replicated 3 times. From 1990 onwards, periodic modifications have been made in all the treatments splitting the plots in two equal halves of $4 \times 3 \text{ m}^2$ leaving one half as original. In the original treatments, recent data revealed that the use of Farm Yard Manure (FYM) @ 10 t ha^{-1} gave significantly ($P \leq 0.05$) higher yield of 2.3 t ha^{-1} in wheat, whereas control plot gave the lowest grain yield of 277 kg ha^{-1} . Similarly, in the modified treatments, the use of FYM @ 10 t ha^{-1} along with inorganic Nitrogen (N) and Potassium oxide (K_2O) @ 50 kg ha^{-1} produced significantly ($P \leq 0.05$) the highest yield of 2.4 t ha^{-1} in wheat. The control plot with an indigenous nutrient supply only produced wheat yield of 277 kg ha^{-1} after 35th year completion of rice-rice-wheat system. A sharp decline in wheat yields was noted in minus N, phosphorus (P), Potassium (K) treatments during recent years. Yields were consistently higher in the $\text{N:P}_2\text{O}_5:\text{K}_2\text{O}$ and FYM treatments than in treatments, where one or more nutrients were lacking. The application of P_2O_5 and K_2O caused a partial recovery of yield in P and K deficient plots. There was significant ($P \leq 0.05$) effect of use of chemical fertilizers and manure on soil properties. The soil analysis data showed an improvement in soil pH (7.8), soil organic matter (4.1%), total N content (0.16%), available P ($503.5 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$) and exchangeable K ($137.5 \text{ kg K}_2\text{O ha}^{-1}$) in FYM applied treatments over all other treatments. The findings showed that the productivity of the wheat can be increased and sustained by improving nutrient through the integrated use of organic and inorganic manures in long term.

Key words: FYM, inorganic, long term, organic and rice-rice-wheat

सारांश

धान-धान-गहुँ वाली प्रणालीमा रासायनिक र प्राज्ञारिक मलको वाली उत्पादन र माटोको उर्वरकतामा पर्न सक्ने दिर्घकालिन असरको शोधकार्य लागी सन् १९७८ देखि राष्ट्रिय गहुँ वाली अनुसन्धान कार्यक्रम भैरहवामा एक परीक्षण शुरु गरिएको थियो। उक्त परीक्षण रेन्डोमाईज्ड कम्प्लीट ब्लक डिजाईनमा तीन पटक छुट्टाछुट्टै गरिएको थियो। सन् १९९० पछि समय अनुसार उपचारहरूलाई दुई बराबर भागमा विभाजन गरिदै लगियो। जस अनुसार 4×3 वर्ग मिटरको दुई बराबर भागमा आधी भागलाई मूल उपचारको रूपमा राखियो। मूल उपचारहरूको हालको आँकडाले गोठेमलको 90 टन प्रति हेक्टरको योगबाट गहुँको उत्पादन 2.3 टन प्रति हेक्टरको पाईयो भने, कुनै पनि मल नराखेको उपचारमा सबै भन्दा कम 277 के जी प्रति हेक्टर उत्पादन भयो। त्यसैगरी, परिमार्जित उपचारमा तत्कालको आँकडा अनुसार गोठेमल 90 टन प्रति हेक्टर र रासायनिक मल 50 के जी नाईट्रोजन र 50 के जी पोटासियम प्रतिहेक्टरको संयुक्त प्रयोगले गहुँको 2.4 टन प्रति हेक्टर उल्लेखनिय बढी उत्पादन भएको पाइयो। धान-धान-गहुँ प्रणालीमा माटोको पोषक आपूर्तिले नियन्त्रित गहुँमा 35 औ वर्ष पछि 277 के जी प्रति हेक्टर मात्र गहुँको उत्पादन भयो। हालका वर्षहरूमा नाईट्रोजन, फस्फोरस र पोटासियम नराखेको उपचारमा गहुँको उत्पादनमा धेरै कमि आयो। एन. पि. के. र गोठेमल राखेको उपचारहरूमा कुनै एक वा एक भन्दा बढी पोषकतत्व नराखेको उपचारमा भन्दा गहुँको उत्पादन निकै बढी थियो। फस्फोरस र पोटासियमको प्रयोगबाट गहुँको उत्पादनमा कमी आएको देखियो। माटोको गुणमा रासायनिक मलको प्रयोग र खादको महत्वपूर्ण प्रभाव थियो। माटोको विश्लेषण आँकडा अनुसार अन्य सबै उपचार संग तुलना गर्दा गोठेमलको प्रयोग गरिएको प्लटहरूमा माटोको पी एच 7.8 , प्राज्ञारिक पदार्थ 4.1% , कुल नाईट्रोजन 0.16 , उपलब्ध फस्फोरस 503.5 र पोटासियम 137.5 किलो प्रतिहेक्टर रहि सुधारात्मक रहेको पाइयो। नेपालको परिप्रेक्षमा प्राज्ञारिक र रासायनिक मलको दिर्घकालिन एकीकृत उपयोगबाट गहुँको उत्पादकत्वमा अभिवृद्धि हुनुका साथै लामो समयको लागि पोषकतत्वमा सुधार हुन सक्ने निष्कर्ष रहेको देखिन्छ।

INTRODUCTION

Rice and wheat mostly grown in sequencing order especially in Terai region represents the most important food crops of Nepal. The Terai plain, meets about 75% of the country's total cereal food demand (NARC 1995). However, productivity and profitability are quite low despite of the fact that a doubling of the crop production in the next 25 years is required to meet Nepal's estimated population growth (NARC 1995, Hobbs and Morris 1996, Kshetri 2010). Increasing the productivity of land through intensive cropping system depletes nutrient reserves of the soil at faster rate (Regmi et al 2003) while unbalanced fertilizer application may disturb nutrient availability to crops, leading to a reduction in yield (Nambiar 1994, Yadav et al 2000). Improving productivity and increasing cropping intensity to sustain yields and meet the future food needs requires adequate soil fertility (Regmi et al 2003). Therefore, to maintain land productivity for crop production, it is likely that new emerging nutrient deficiency or imbalances are addressed, identified and corrected promptly. In such sequence, identification of micronutrients zinc deficiency in rice and boron in wheat represents the best examples (Regmi et al 2003).

It has been observed that continuous use of chemical fertilizers in imbalanced form deteriorates soil physical properties (Biswas et al 1971, Prasad et al 1983, Bhattacharyya et al 2015). Detrimental effects of chemical fertilizers even in balanced form on soil physical properties are also being observed. For example, decline in soil organic carbon and associated decline in system productivity under rice-wheat system with the long-term use of recommended NPK was observed in some field studies (Nambiar 1994, Abroal et al 2000, Yadav et al 2000). The widespread stagnation and occasional decline in rice-wheat productivity over the last about three decades have become a matter of serious concern, as rice-wheat is the major cropping system in south Asia, feeding more than 400 million people world-over (Ladha et al 2003).

Soil fertility is largely attempted to maintain by application of compost and manure, but in recent years a decline in soil fertility has been reported (Shrestha et al 2000). There has been considerable research in Nepal on soil fertility enhancement and soil and water conservation techniques over the years (Keatinge et al 1999, Acharya et al 2000). Despite of that the soil fertility in long run in relation to cropping pattern has always been a subject of interest because of several factors associated including the manure and fertilizer use. Therefore, long-term experiment has always been suggested to be valuable for evaluating the effects of continuous cropping on the capacity of a system to sustain nutrient supply and the productivity (Regmi et al 2003).

For recommending appropriate technologies to maintain soil fertility as well as the environment, it is necessary to evaluate long-term effects of inorganic and organic manures on soil properties (Gami and Sah 1988, Bhattacharya et al 2015). The long-term soil fertility experiments are valuable for understanding the relationships among changing soil, crop management practices and productivity (Bhattacharya et al 2015). It is also important that the data collected from constantly monitoring long-term experiment could be useful for improving statistical and simulation tools (IRRI 2000, Bhattacharayya 2015). Therefore, a long term experiment was initiated under rice-rice-wheat system to evaluate the effects of long term application of mineral fertilizer and organic manure on soil properties and wheat grain yield under rice-rice-wheat system.

MATERIALS AND METHODS

Experimental Site, Treatments and Crop Management

The long term experiment was started in 1979/80 at National Wheat Research Program located in Bhairahawa, Rupandehi District in the western Terai Region of Nepal at the latitude of 27°32' and the longitude of 83°28' with an elevation of 120 masl. In this site, the air temperature ranges from a minimum of about 7°C in winter to the maximum of about 45°C in summer. In general, the site receives ample rainfall during the monsoon, which starts from June and continues up to September. The mean annual rainfall is about 1800 mm. The soil of the experiment plot was silty loam with a pH of 8.0, organic matter (OM) of 1.783%, P of 9.75 u/g, exchangeable K of 126 ug/g soil and bulk density of 1.6 g cm⁻³ with hard pan just below the plow layer. The soils in the experiment area are classified as Typic Heplaquepts.

The experiment was laid out in randomized complete block design with 9 treatments which were replicated 3 times. The plot size was 6 x 4 m² up to 1990. From 1990 onwards, periodic modifications have been made in all the treatments splitting the plots in two equal halves of 4 x 3 m² leaving one half as original (Table 1). Wheat was sown in rows of 25 cm apart. Farm yard manure was applied at 7-10 days before seeding. Half dose of N and full dose of P and K were applied as basal. Remaining 50% nitrogen was top dressed at 21-25 days after seeding in wheat.

Measurement of Crop Parameters

Table 1. Original and modified treatments of LTSFE (R-R-W)

Tr No	Original Treatment N, P ₂ O ₅ , K ₂ O kg/ha	Modified Tr. (1991) N, P ₂ O ₅ , K ₂ O kg/ha	Modified Tr.(1995 onward) N, P ₂ O ₅ , K ₂ O kg/ha
1	0: 0:0 - R & W	0: 0:0 - R & W	100:50:100- R & W
2	100:0:0- R & W	100:30:30 - R 100:40:30- W	100:30:30 - R 100:40:30- W
3	100:30:0 - R 100:40:0 - W	100:30:0 - R 100:40:0 - W	100:30:30- R 100:40:30 - W
4	100:0:30- R & W	100:100:30 One time - ER	100:30:30 - R 100:40:30 - W
5	100:30:30 - R 100:40:30 - W	100:30:30 - R 100:40:30 - W	100:30:100 - R 100:40:100 - W
6	100:0:0 - R 100:40:30 - W	100:30:0 - ER	100:0:0 - R 100:40:30 - W 100:30:0 - ER
7	50:0:0 - R & W+ 30 cm stubble incorporation	50:20:0 - R & W +30 cm stb. incorporation	50:20:0 - R & W +30 cm stb. incorporation
8	50:20:0 - R & W+ 30 cm stubble incorporation	50:20:0 - R & W+ 30 cm stubble incorporation	50:20:20 - R & W + 30 cm stubble incorporation
9	F Y M 10 t/ha - R & W	F Y M 10 t/ha + 50 kg N - R & W	F Y M 10 t/ha + 50 kg N + 50 kg K20 - R & W

LTSFE= Long Term Soil Fertility Trial, Tr= Treatments

Soil Sampling and Analysis

Soil samples were collected from each of the experimental plots after harvesting wheat (5 April 2014). Each soil sample was randomly collected from the 0 to 20 cm deep plough layer using an auger. For this, the air-dried samples were crushed and passed though a 2 mm sieve. Soil pH was determined by a pH meter after extraction from a soil: water ratio of 1:2. Organic matter was determined using the Walkley and Black dichromate method (Nelson and Sommers 1982) and total N using Kjeldhal's method (Bremner and Mulvaney 1982) For available P determination modified Olsen's (Olson and Sommers 1982) methods used; exchangeable K was estimated by 1M ammonium acetate extraction (Knudsen et al 1982) followed by flame photometric determination.

Statistical Analysis

Recorded data were compiled and tabulated in Ms-Excel. Data for each parameter over two year's period was subjected to analysis of variance using a Randomized Complete Block Design according to MSTATC (Steel and Torrie 1980). Treatment means were compared using least significant difference (LSD) test at $P \leq 0.05$.

RESULTS

Grain yields of wheat in rice-rice-wheat system were affected by the application of different combination of manures and fertilizer treatments.

Original Treatments

The various growth and yield attributing parameters significantly ($P \leq 0.05$) differed with the varied use of organic and inorganic sources of nutrients in wheat crop. The plant height, productive tillers per m^2 , test weight, straw and grain yields were significantly ($P \leq 0.05$) higher where use of FYM @10 t ha^{-1} were applied with the highest yield of 3616 kg ha^{-1} in 2011 and 2383 kg ha^{-1} in 2012 against 425 kg ha^{-1} and 277 kg ha^{-1} in control (T1), respectively (Appendix 1, 2 and 3). In 2012/13, logging during heading stage of wheat caused the low yield.

Table 2. Grain yield (kg ha^{-1}) of wheat in LTFT during 2011/12 and 2012/13

Trt	2011/12		2012/13	
	Original	Modified	Original	Modified
T1	425	3278	277	1793
T2	1218	1865	370	1521
T3	1582	2124	333	1538
T4	513	3176	370	1682
T5	2622	3348	1325	1899
T6	2612	2808	1205	1418
T7	386	1695	362	1085
T8	1707	2524	1064	1709
T9	3616	3799	2278	2383
F test	***	**	***	*
CV, %	25.9	21.5	16.6	16.4
LSD (0.05)	1296	1015.8	223.5	462.2
F test	***	**	***	*

LTFT= Long Term Fertility Trial, Trt= Treatments

Modified Treatments

The highest grain yield (3799 kg ha^{-1} in 2011/12 and 2383 kg ha^{-1} in 2012/13) was occurred in treatment-T9 ie FYM @10 t ha^{-1} + N @50 kg + 50 kg K_2O , where as application of N: P_2O_5 : K_2O @50:20:0 plus 30 cm stubble incorporation gave the lowest grain yield of wheat (1695 kg ha^{-1} in 2011/12 and 1085 kg ha^{-1} in 2012/12) probably due to absence of K in the treatment (Table 2). This alarms situation in farmers' field where farmers generally do not apply potassium fertilizer in wheat crop.

Yield Trend of Wheat

The data revealed that the grain yield of wheat was higher in treatment T9M (FYM 10 t ha^{-1} and N and K_2O 50 kg ha^{-1} each which is followed by FYM application alone (T9) and the recommended fertilizer dose (T5). The results showed that yield trends of wheat for N treatment (T2), NK treatment (T4) and NP treatment (T3) were similar to the control (no-fertilizer) by end of 35 years of the experiment (Figure 1, 2 and 3).

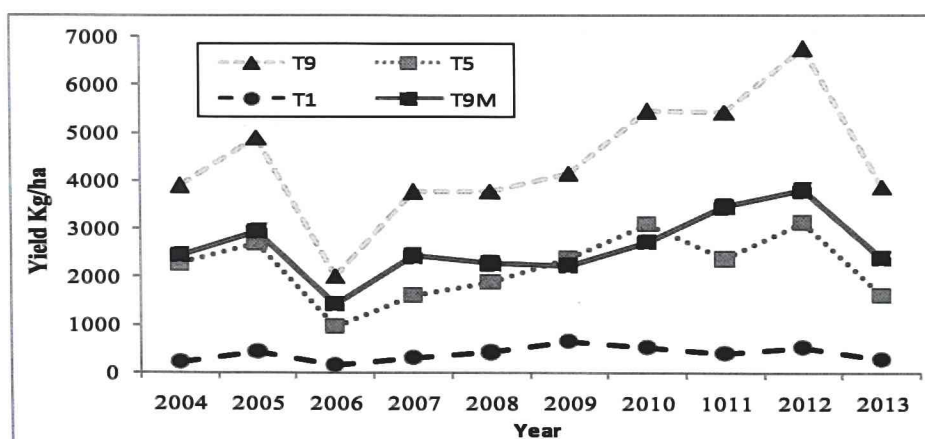


Figure 1. Grain yield trend of long-term application of fertilizers and organic manure on wheat.

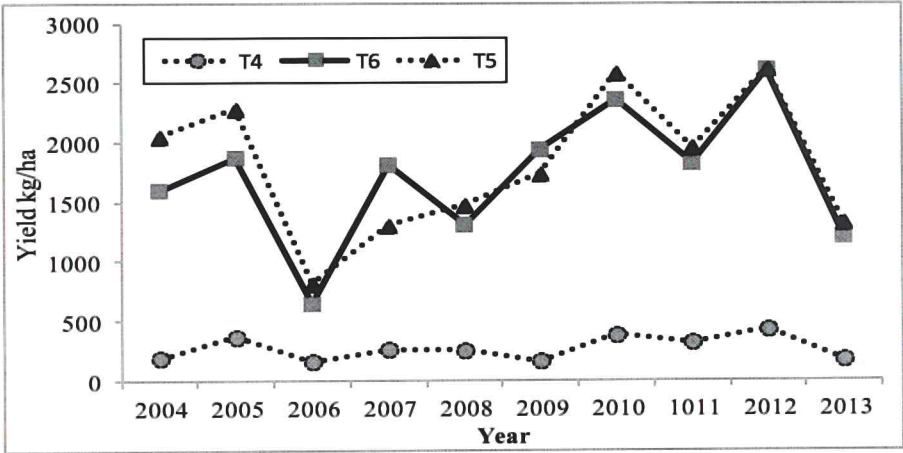


Figure 2. Effect of P addition on grain yield of wheat.

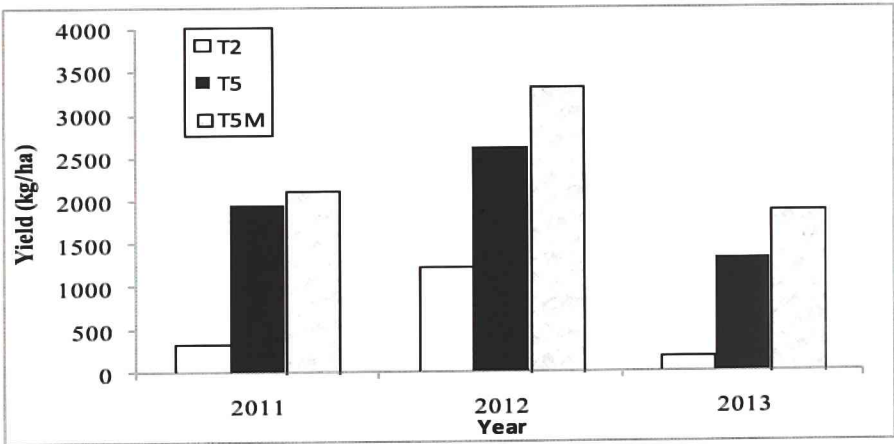


Figure 3. Effect of P and K omission on grain yield of wheat.

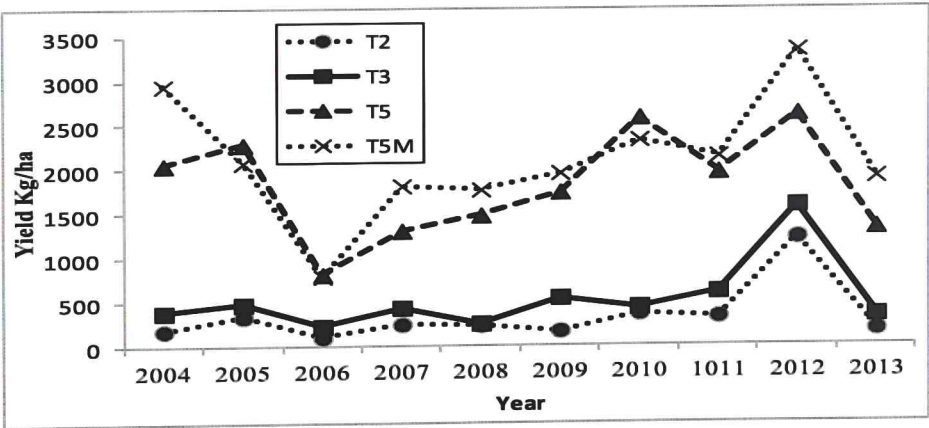


Figure 4. Addition of K increases wheat yield.

Soil Fertility Status

There was a significant ($P\leq0.05$) effect of manures and fertilizers on soil pH, soil organic matter, N content, available P and exchangeable K. At harvest of wheat crop, soil pH was improved in T9 (7.87) from the application of farm yard manure as compared to original (8.0) and significantly ($P\leq0.05$) higher SOM (4.12%) was obtained by applying the FYM and inorganic fertilizer which was statistically similar with the application of FYM alone (4.10%) (Table 3). Similarly, significantly ($P\leq0.05$) higher soil N content was

obtained from the combined application of FYM (0.16%) but it was similar to the application of FYM plus chemical fertilizer (N and K₂O) and it was the lowest (1.13%) in control plot (0.8 %).

At the end of 35 years experiment, the highest soil available P of 503.5 kg ha⁻¹ was occurred in the plots applied with FYM, while the lowest available soil P of 11.5 kg ha⁻¹ was in the control plot. Similarly, exchangeable K was significantly ($P \leq 0.05$) higher (137.8 kg ha⁻¹) with N and K₂O which was at par with the application of FYM alone (117.1 kg ha⁻¹). The soil analysis data showed an improvement in soil pH (7.8), soil organic matter (4.1%), total nitrogen content (0.16%), available P (503.5 P₂O₅ kg ha⁻¹) and exchangeable K (117.1 K₂O kg ha⁻¹) in FYM applied treatments over all other treatments.

Table 3. Soil chemical properties affected by organic & chemical fertilizers NWRP, Bhairahawa, Nepal

Treatment	pH		Organic matter (%)		Nitrogen (%)		P ₂ O ₅ (kg ha ⁻¹)		K ₂ O (kg ha ⁻¹)	
	Original	Modified	Original	Modified	Original	Modified	Original	Modified	Original	Modified
T ₁	8.25	8.11	1.27	1.89	0.08	0.09	11.5	123.8	94.3	117.1
T ₂	8.25	8.18	1.67	2.18	0.09	0.10	11.7	50.0	76.0	80.6
T ₃	8.16	8.17	2.15	2.33	0.10	0.11	93.7	70.5	34.9	76.0
T ₄	8.23	8.14	1.67	1.89	0.09	0.09	14.7	40.8	103.4	53.2
T ₅	8.21	8.16	2.30	2.39	0.11	0.11	44.4	57.2	71.5	103.4
T ₆	8.20	8.21	2.15	1.65	0.10	0.09	10.7	34.0	62.3	62.3
T ₇	8.23	8.17	2.02	2.13	0.10	0.10	12.3	28.8	76.0	66.9
T ₈	8.18	8.15	2.06	1.86	0.10	0.93	38	34.4	71.5	76.0
T ₉	7.87	7.95	4.10	4.12	0.16	0.16	503.5	403.7	117.1	137.8
F-test	**	Ns	***	**	***	**	***	***	*	*
LSD (0.05)	0.16	-	0.89	0.99	0.03	0.03	45.63	49.86	40.57	40.67
CV, %	1.1	1.1	23.9	25.2	14.7	15.8	32.1	30.8	29.8	28.4
Initial (1978/79 AD)	8.0		1.025		0.088		9.8		126	

***, ** and * denotes significant at 0.1%, 1% and 5% level of significance respectively and Ns stands for non significant

LTF= Long Term Fertility Trial

DISCUSSION

Soil fertility and plant nutrient management are key issues to be addressed to understand the reasons for declining crop yields. After 35 years of the experiment, significantly ($P \leq 0.05$) higher yield of wheat was found in T₅, T₉ and T_{9M} as compared to other treatments (Figure 1). There was fluctuation in grain yield of wheat which could be due to variation in rainfall, temperature, moisture in general during crop growing period. There was very low grain yield in all P missing treatments (T₁, T₂, T₄, and T₇) (Figure 1, 2 and 3). This shows P is one of the most limiting factors for wheat crop. In all treatments in which one or more primary nutrients were lacking, resulted decline in wheat grain yield. This finding was similar to many other studies earlier reported (Dawe and Dobermann 1999, Hobbs and Morris 1996). These implying either the changes in biochemical and physical composition of soil organic matter (SOM) might resulted in gradual decline in the supply of soil nutrients to crop due to inappropriate fertilizer applications causing nutrient imbalances (macro and micro) (Paroda et al 1994).

The grain yield of wheat was found higher in T₅ (100:30:30 – R and 100:40:30 – W) followed by T₆ (100:0:0 – R and 100:40:30 – W). The significant ($P \leq 0.05$) differences in grain yield were seen between the N: P₂O₅: K₂O and N: P₂O₅ treatments, indicating that the yield reductions in this experiment were also due to the K deficiency. With the increase in K level, there was increase in the grain yield of normal wheat (Figure 4).

Proper use of chemical fertilizers and organic manures supports increased agricultural productivity and at the same time, helps maintain soil fertility (Gami and Sah 1988). Similar results have been revealed in present study. The results showed that neither the present dose of N: P₂O₅: K₂O nor FYM can sustain productivity in such system. These results corroborate those of Flinn and De Datta (1984), who reported a yield decline under the full recommended dose of fertilizer. In many fertilizer experiments, Nambiar and Abrol (1989) have also found a declining trend with adequate NPK. FYM alone could not supply N and K requirement of wheat crop. The yield increase with the modified (T_{9M}) possibly resulted from replacement of the original (T₉) due to balance dose of N: P₂O₅: K₂O and other micronutrients contained in FYM.

In the long run, soil fertility is not sustained from the balanced fertilizer application alone while application of FYM helps to boost up crop yield (Lal and Mathur 1989, Kabeerthumma et al 1993) and improve physical soil status (Lal and Mathur 1989b, Kumar and Tripathi 1990). Declining yield trend at lower N level over the years may indicate the diminishing supply capacity of soil. The single greatest cause of declining crop production is unbalanced fertilization (Rattan and Singh 1997). Unbalanced fertilizer application has led to a chronological emergence of macronutrients such as phosphorus and potash (P and K) and micronutrients such as zinc, sulfur and manganese (Zn, S and Mn) deficiencies. Even balanced application of macronutrients devoid of organic materials has been implicated in the deterioration of the physical, chemical and biological health of soil (Rattan and Singh 1997).

Our findings also showed that the regular application of FYM might significantly increase the available phosphorus, total nitrogen and carbon contents in the soil but not the potassium level (Table 3). The five-year long-term experiment clear showed that the recommended chemical fertilizer dose of 100:40:30 (N: P₂O₅: K₂O) kg ha⁻¹ for wheat crop seems to be inadequate for boosting yield and soil productivity. Long-term application of FYM @ 10-tons ha⁻¹ was found to enhance its yield and increased the soil nutrients content in the soil. Balanced application is considered a sustainable way of enriching soil and hence restoring its fertility over time (Bhattachryya et al 2015). Similar long term fertility experiments showed that depletion of soil nutrients caused by years of intensive cropping is a common feature that appears to contribute to the observed yield decline (Regmi 1996, Yadvinder-Singh et al 2000). In

addition to mining of nutrients, there is some evidence of changes in the availability of nutrients to the plant. In most rice-wheat experiments, the soil organic matter declines over time, but, it is not only the decline per season; there may also be changes in the chemical composition of organic matter (Olk et al 1996, 1998, Bronson and Hobbs 1998) that influence the capacity to supply nutrients to the plant. A sustainable fertilizer management strategy must ensure high and stable overall productivity and sufficient nutrient supply for potential yield increases.

The control plot with an indigenous nutrient supply only supported wheat yield of 277 kg ha⁻¹ in long term experiments of rice-rice-wheat system (Figure 1). FYM alone could not supply N and K requirement of wheat crop. The increase in wheat yield was observed due to the application of 50 kg ha⁻¹ both N and K₂O to FYM treatment. The lowest grain yield of wheat may be due to absence of potassium in the treatment. This alarms situation in farmers' field where farmers generally do not apply potassium fertilizer in wheat crop. The soil analysis data showed an improvement in soil pH, soil organic matter, total nitrogen content, available phosphorus and exchangeable potassium in FYM applied treatments over all other treatments. Declining yields and soil nutrient balance in a long-term rice-rice-wheat study suggest depletion of soil potassium (K) and P fertilization seem to be primary reasons for limited and declining crop yields. The findings showed that the productivity of the wheat can be increased and sustained by improving nutrient through the integrated use of organic and inorganic manures in long term.

ACKNOWLEDGMENTS

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APPENDIXES

Appendix 1. Rating chart of soil values to determine the fertility status of experimental soil

Nutrient	Low	Medium	High
Available N (%)	<0.10	0.1-0.2	>0.2
Available P ₂ O ₅ (kg/ha)	<30	30-55	>55
Available K ₂ O (kg/ha)	<110	110-280	>280
Organic matter (%)	<2.5	2.5-5.0	>5.0
pH	<6.0 (Acidic)	6.0-7.5 (Neutral)	>7.5 (Alkaline)

Source: Khatri Chettri 1991 and Jaishy 2000

Appendix 2. Plant height, productive tillers/m², 1000-grain weight, straw yield and grain yield of wheat in LTFT conducted at NWRP, Bhairahawa, 2011/12

Trt	Plant height, cm		Tillers/m ² (n)		1000-grain weight (g)		Straw yield (kg ha ⁻¹)		Grain yield (kg ha ⁻¹)	
	Original	Modified	Original	Modified	Original	Modified	Original	Modified	Original	Modified
T ₁	66.33	100.87	187.7	220.3	30.53	42.40	1722	7611	425	3278
T ₂	59.87	93.20	167.3	225.0	23.07	38.75	1389	6778	1218	1865
T ₃	91.77	87.80	237.3	214.3	35.73	32.73	6389	5222	1582	2124
T ₄	64.90	96.47	206.3	255.3	29.20	37.07	1722	7389	513	3176
T ₅	94.70	99.47	229.7	234.3	36.87	41.57	6389	7389	2622	3348
T ₆	94.13	95.10	237.3	230.0	40.40	38.13	6222	6444	2612	2808
T ₇	60.90	83.03	163.3	221.3	26.33	35.47	1278	3444	386	1695
T ₈	81.87	92.37	199.0	230.7	31.00	41.07	3500	4778	1707	2524
T ₉	97.53	100.03	228.0	239.7	44.07	45.33	8167	8833	3616	3799
F test	***	**	**	Ns	***	**	***	***	***	**
CV, %	4.4	5.0	10.8	12.1	7.0	8.1	14.1	13.6	25.9	21.5
LSD	6.032	8.237	38.71	48.36	3.986	5.519	997.4	1510.4	1296	1015.8

***, ** and * denotes significant at 0.1%, 1% and 5% level of significance respectively and Ns stands for non significant
LTFT= Long Term Fertility Trial, GY= Grain Yield, Trt= Treatments

Appendix 3. Plant height, productive tillers/m² test weight, straw yield and grain yield of wheat per hectare in LTFT conducted at NWRP, Bhairahawa, 2012/13

Trt	PH		spikes/m ²		1000-grain wt.		Biomass		Grain yield (kg ha ⁻¹)	
	Original	Modified	Original	Modified	Original	Modified	Original	Modified	Original	Modified
T ₁	65.00	91.33	144	176.7	28.81	32.48	1111	4389	277	1793
T ₂	58.00	83.67	163	207.3	25.27	26.29	889	4000	370	1521
T ₃	66.67	84.44	134	196.0	19.81	27.36	1278	4278	333	1538
T ₄	59.67	83.67	130	180.0	30.14	28.23	1000	43.89	370	1682
T ₅	82.67	91.67	205	179.7	31.00	30.17	3778	6944	1325	1899
T ₆	83.33	82.00	171	161.7	27.79	27.42	3444	3278	1205	1418
T ₇	55.67	77.00	132	183.3	27.19	25.99	889	2878	362	1085
T ₈	84.67	89.67	156	221.3	27.07	31.92	2556	3711	1064	1709
T ₉	90.00	95.67	215	220.3	38.19	30.77	6000	6600	2278	2383
F-test	***	**	**	Ns	***	Ns	***	Ns	***	*
CV, %	4.4	3.1	16.3	17.0	7.2	9.2	11.5	33.9	16.6	16.4
LSD	5.41	4.64	45.51	56.40	3.55	4.63	462.0	3351.7	223.5	462.2

***, ** and * denotes significant at 0.1%, 1% and 5% level of significance respectively and Ns stands for non significant
LTFT= Long Term Fertility Trial, Trt= Treatments

Management of Anthracnose in Soybean using Fungicide

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ABSTRACT

Experiments on soybean (*Glycine max* L. Meril) were carried out aiming to control anthracnose (pod blight) caused by fungus, *Colletotrichum truncatum* with five treatments represented by different fungicidal sprays against control receiving no spray with three replicates of each under field conditions during two consecutive years from 2012 to 2013. In 2012, the higher Percent Disease Control (PDC) and Percent Yield Increase (PYI) were estimated in plot treated with SAAF (Carbendazim 12% + Mancozeb 63%) followed by Mancozeb fungicides. The mean Pod Infection (PI) was low in plots treated with SAAF followed by Mancozeb. Almost similar trends of disease control were observed in 2013. The lower Percent Disease Index (PDI) was 46.25% and mean PI was 29.67% with higher yield value of 2431.25 kg/ha obtained from the plots sprayed with SAAF then by Mancozeb. The results showed that, the combined treatment with fungicides, SAAF followed by Mancozeb were effective to control anthracnose or pod blight disease of soybean to increase the yield.

Key words: Anthracnose, fungicides, pod blight, soybean

सारांश

भटमास बालीमा कोलेटोट्रिचम ट्रंकटम नामक हुँसीले लाग्ने कोसा डड्ने रोग व्यवस्थापन गर्ने उद्देश्यले रामपुर चितवनमा सन् २०१२ देखि र २०१३ मा बजारमा उपलब्ध विभिन्न हुँसीनाशक विषादीहरूको रोग नियन्त्रण प्रभावकारिता परीक्षण गरिएको थियो। सन् २०१२ को वर्षायाममा हुँसीनाशक विषादी साफ कारबेन्डाजिम १२% + म्यान्कोजेब ६३% बाट उपचारित प्लटहरूमा रोग नियन्त्रण प्रभावकारिता ४२.९९% र उत्पादनमा वृद्धि १०८.६९% रहेको र त्यसपछि म्यान्कोजेबद्वारा उपचारित प्लटमा पनि केही पनि नछरेको प्लटभन्दा उच्च रहेको पाइयो। कोसामा देखिने रोगको संक्रमणको औषत प्रतिशत पनि क्रमशः साफ र म्यान्कोजेब द्वारा उपचारित प्लटमा क्रमशः कम (२२.३९% र २३.०४%) रहेको पाइयो। सन् २०१३ को परिक्षणमा पनि अघिल्लो वर्षको रोग व्यवस्थापनको आंकडा जस्तै नतिजा प्राप्त भयो। सबैभन्दा कम रोग र कोसामा रोगको संक्रमणको औषत प्रतिशत साथै उच्च उत्पादन क्रमशः ४६.२५%, २९.६७% र २४.३९.२% के.जी. प्रति हेक्टर साफ द्वारा उपचारित प्लटमा पाइयो भने, त्यसपछि म्यान्कोजेब द्वारा उपचारित प्लटमा थियो। यसर्थ, दुई वर्षको परिक्षणको नतिजा अनुसार हुँसीनाशक विषादीहरू साफ र म्यान्कोजेब भटमासको कोसा कुहिन रोग व्यवस्थापन साथै उत्पादन बृद्धिमा प्रभावकारी रहेको पाइयो।

INTRODUCTION

Soybean (*Glycine max* L. Merrill) is an important summer legume of mid hill, grown as an intercrop with maize or in paddy bund that occupies about 80% in terms of total soybean area and production in Nepal (GLRP 2012). Soybean occupies an area of about 24934 ha with total production of 29221 mt with an average productivity of 1172 kg/ha (MoAD 2013), which is far less than global average productivity of 1.712 tons/ha (Masuda and Goldsmith 2008). For poor productivity prevailing diseases are one of the main reasons in Nepal. It is known that more than hundred pathogens affect soybean crop, some of them causing 10-12% yield losses (Mittal et al 1993). Among those Anthracnose disease caused by *Colletotrichum truncatum* in soybean is one of the most important seed-borne fungal pathogens appears late in season on stem, pods and seeds (GLRP 2011). The disease causes reduction of seed germination, seed quality and severe yield losses may occur in warm and humid regions of tropics and subtropics (Sinclair 1989) where soybean production is presently expanding.

In India, anthracnose is considered the most serious soybean disease (Khare and Chacko 1983). In the southern United States, this disease caused estimated yield losses ranging from 0.1 to 7.0% in the growing seasons (Koldenhoven et al 1983, Mulrooney 1985, 1986, 1988). Estimates of maximal seed yield reduction to anthracnose ranged from 16 to 26% in susceptible cultivars in Alabama (Backman et al 1982). Yield losses of 30 to 50% were reported in Thailand and 100% in India (Sinclair 1989).

Although several soybean lines were rated as resistant to the pathogen, no germplasm accessions tested so far have been shown to be immune (Manandhar et al 1988). *C. truncatum* is seed-borne and seed-transmitted disease which might cause systemic infection (Neergard 1979). The pathogen over-winter in crop debris (Athow 1973, Sinclair 1989) and several weed species may also serve as sources of inoculums (Hartman et al 1986, McLean and Roy 1988). Management of plant disease successfully achieved through application of chemical fungicides. Although, pod blight in soybean results in severe yield losses, however, very few works with respect to disease management have been carried out to cope of this disease in Nepal. So, the present experiment was undertaken to evaluate efficiency of available fungicides in market against soybean anthracnose disease under field conditions.

MATERIALS AND METHODS

The experiments were conducted during summer of 2012-2014 at Grain Legumes Research Program, Rampur, Chitwan by laying out in a well managed piece of land using Randomized Complete Block Design having five treatments with different fungicides against one control with four replicates of each. During soybean season, a susceptible variety Ransom was planted on second week of July in a unit plot size of 3m x 2m and 50 cm x 10 cm spacing. The fungicides were Dithane M-45 (Mancozeb 75% WP) @ 2.5 g/l, Krilaxyl (Metalaxyl

8%+ Mancozeb 64% WP) @ 2 g/l, Bavistin (Carbendazim 50% WP) @ 2g/l, Blitox-50 (Copper oxy chloride 50% WP) @ 3 g/l and SAAF (Carbendazim 12% + Mancozeb 63%) @ 2.5 g/l.

After completion of the sowing, the plots were kept under constant supervision from sowing to harvest. Agronomic practices were followed as recommended. First spray was given just after the appearance of disease symptom in the field. Three sprays were given at an interval of 15 days. Data were recorded before every spray using 1-9 scoring scale on 5 randomly tagged plants/plot. The control plots received no fungicides. Percent Disease Index (PDI) was computed according to the formula (Wheeler 1969) and calculation was based on the final data recorded at 15 days after the last spray.

Percent Disease Control (PDC) was calculated on the basis of the formula developed by Shivankar and Wangikar (1993). Early Plant Stand (EPS) and Final Plant Stand (FPS) were recorded. At harvest, data on total number of pod per plant, number of infected and healthy pods, 100 seed weight and seed yield were recorded with yield data presented in terms of hectare. Yield increase over the control was calculated. All data were analyzed statistically using MSTAT-C computer package program. Treatment means were compared using Least Significance Difference (LSD) and Duncan's Multiple Range Test (DMRT) at 5% levels of significance. The correlation among percent yield increased over control and percent disease control was calculated.

RESULTS

Efficacy of Fungicides In-vivo

The lower PDI with a value of 42.53% was recorded in plot treated with SAAF. The second most effective 47.25% of PDI was occurred in Mancozeb treated plants, while and highest value of 73.57% was in control. Final plant stand was also noticed higher in plot treated with SAAF in 47.25% while 45.75% in plots treated with Mancozeb. In SAAF treated plot the Percentage Disease Control (PDC) and Percent Yield Increase (PYI) were 42.19% and 108.69%, respectively followed by Mancozeb compared to control plot. The mean Pod Infection Percentage was noticed lower with a value of 22.31% in plot treated with SAAF followed by Mancozeb with 23.04% of value (Table 1).

Table1. Effect of fungicides on pod blight disease severity and yield performance of soybean at Rampur, Chitwan, Nepal in 2012/13

Treatments	Early Stand	PDI (%)	Pod/ plant	Final Stand	Yield Kg/ha	HSWT (g)	PI (%)	PDC (%)	PYI (%)
SAAF (2.5g/l of water)	50.25	42.53 ^c	51.50 ^a	47.25 ^a	1500.00 ^a	15.45 ^a	22.31 ^d	42.19	108.69
Mancozeb (2.5g/l of water)	46.75	47.25 ^c	48.50 ^{ab}	45.75 ^{ab}	1302.08 ^b	14.62 ^b	23.04 ^d	35.77	81.16
Krilaxyl (2g/l of water)	51.50	59.40 ^b	45.25 ^b	41.75 ^{ab}	1000.00 ^c	13.44 ^d	33.59 ^{bc}	19.26	39.13
Blitox-50 (3g/l of water)	46.50	48.60 ^c	47.50 ^b	42.75 ^{ab}	1062.50 ^c	13.97 ^c	32.82 ^c	33.94	47.83
Bavistin (2g/l of water)	42.75	68.62 ^a	32.00 ^c	40.00 ^{bc}	864.58 ^d	13.27 ^d	38.76 ^b	6.73	20.29
Control	51.25	73.57 ^a	25.25 ^d	34.00 ^c	718.75 ^e	12.41 ^e	73.92 ^a		
CV, %	12.40	8.73	5.71	10.37	7.81	1.17	9.93		
LSD (0.05)		7.46	3.58	6.55	126.42	0.24	5.60		

Means in column with same superscript is not significantly ($P < 0.05$) different by DMRT (Duncans Multiple Range Test). PDI- Percent Disease Index, FPS – Final Plant Stand/Plot, Yield (Kg/ha) - Grain yield in Kilogram per hectare, HSWT (gm) - Hundred Seed Weight in gram, PI% - Mean pod infection percentage, PDC - Percent Disease Control, YI - Yield Increase, g/l - Gram per litre

Relationship between Percent Disease Control (PDC) and Percent Yield Increase (PYI)

In year 2013, the trends of disease control and yield observations were almost similar comparing to previous year. The PDI, number of pods per plant, final stand, yield, 100 seed weight and mean pod infection were significantly ($P < 0.05$) varied with the treatments. The lower (46.25%) PDI was recorded on plots sprayed with SAAF, the values increased when treated with Mancozeb having a value of 53.42% and then others. The maximum Mean Pod Infection percentage of 75.94% was recorded in control plot, while minimum in SAAF treated plot having a value of 29.67% and then in plot treated with Mancozeb (with a value of 33.15%), respectively. The yield of 2431.25 kg/ha and seed weight of 13.52 g/100 were also significantly ($P < 0.05$) higher in plots treated with SAAF and then by Mancozeb sprayed plots. The lower yield was recorded from control plot (743.75 kg/ha) (Table 2).

A linear negative correlation between yield and PDI was observed representing the best fit having $R^2 = 77\%$ (Figure 2). Obviously the yield decreased with the increase in Percent Disease Index (PDI). The linear correlation between PDC and PYI showed positive correlation ($R^2 = 0.796$) (Figure 1) showing that control in disease enhance the yield.

Table 2. Influence of fungicides on pod blight disease severity and yield performance of soybean at Rampur, Chitwan during 2013/14

Treatments	Early Stand	PDI (%)	Pod/ plant	Final stand	Yield kg/ha	HSWT (g)	PI %	PDC (%)	PYI (%)
SAAF (2.5 g/l of water)	147.25	46.25 ^d	48.90 ^a	149.00 ^a	2431.25 ^a	13.52 ^a	29.67 ^b	37.13	226.89
Mancozeb (2.5 g/l of water)	153.75	53.42 ^c	46.07 ^{ab}	139.50 ^b	1522.06 ^b	12.60 ^b	33.15 ^b	27.38	104.64
Krilaxyl (2 g/l of water)	168.50	54.22 ^c	43.42 ^{bc}	131.75 ^c	1430.31 ^d	11.65 ^c	35.71 ^b	26.30	92.31
Blitox-50 (3 g/l of water)	169.75	53.58 ^c	45.25 ^{abc}	134.00 ^{bc}	1477.19 ^e	12.50 ^b	34.04 ^b	27.17	98.61
Bavistin (2 g/l of water)	176.25	65.07 ^b	41.97 ^c	128.50 ^c	1373.55 ^e	11.49 ^c	36.21 ^b	11.55	84.67
Control (Untreated check)	158.00	73.57 ^a	34.00 ^d	107.75 ^d	743.75 ^f	9.38 ^d	75.94 ^a		
CV, %	9.04	4.01	5.52	2.93	1.59	1.91	18.34		
LSD (0.05)		3.48	3.60	5.82	35.86	0.34	11.28		

Means in column with same superscript is not significantly ($P < 0.05$) different by DMRT (Duncans Multiple Range Test) PDI- Percent Disease Index, FPS – Final Plant Stand/plot, Yield (kg/ha)- Grain yield in Kilogram per hectare, HSWT (g)- Hundred Seed Weight in gram, PI% - Mean pod infection percentage, PDC- Percent Disease Control, YI- Yield Increase.

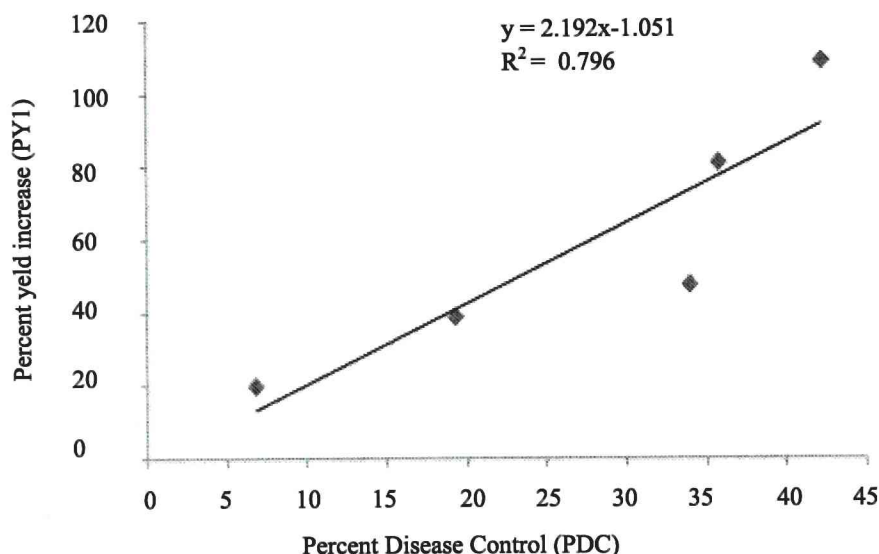


Figure 1. Relationship between PDC and PYI of fungicides used in soybean anthracnose.

DISCUSSION

Chemical treatment is one of the most recommended methods used for plant disease management. The present experiments with various available chemicals on Soybean crop for two consecutive years clearly showed low Percent Disease Index (PDI) and higher yield from the plot treated with SAAF (Carbendazim 12% + Mancozeb 63%) @ 2.5 g per litre of water comparing to control. This findings is in close agreement with Chakraborty and Shyam (1988) and (Ghawde et al 1996), who showed that fungicide Carbendazim was inhibitory to the *Colletotrichum lindemuthianum* causing anthracnose to French bean, *C. truncatum* The fungi *Colletotrichum graminicola* and *C. capsici* cause blight in bitter gourd (Singh and Dwivedi 2002, Dubey and Ekka 2003) while, *C. gloeosporioides* causes anthracnose in mango (Kumar et al 2003). Application of fungicides to control anthracnose disease of soybean reduces the yield losses (Backman et al 1982). Efficacy of fungicides in controlling anthracnose disease and increasing the yields were reported earlier (Khare et al 1972, Chaudhary 1977, Palarpawar and Ghurade 1989, Kumar and Mukhopadhyay 1990, Bhardwaj and Thakur, 1991, Shukla and Singh 1993, Dubey and Ekka 2003, Ekbote 2005).

It is clear that soybean sprayed with fungicides had lesser disease severity with increase in yield than untreated ones. The SAAF and Mancozeb @ 2.5 g/l of water were found best among the sprayed fungicides. The lesser percent disease index, lower mean pod infection percentage along with the higher yield and 100 seed weight was found from the plot applied with SAAF followed by Mancozeb. It is recommended that soybean field should be sprayed by SAAF @2.5 g/l of water twice at vegetative and pod formation stage against anthracnose/pod blight disease for maximum yield with the reduction of disease severity.

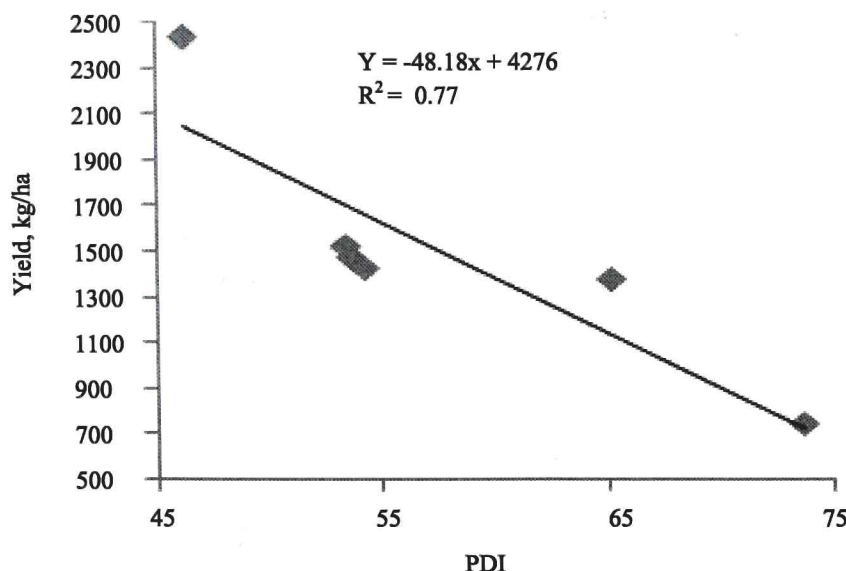


Figure 2. Relationship between Percent Disease Increase (PDI) and yield of soybean.

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Yield Interactions of Wheat Genotypes to Dates of Seeding in Eastern Mid Hills of Nepal

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ABSTRACT

Wheat (*Triticum aestivum* L.) is one of the major cereal crops and staple food sources in Nepal. Wheat varieties being popular in mid hill regions are still in the early stages of adoption. Identification of appropriate date of seeding plays important role in enhancing the adoption rate ensuring the sustainable production. Therefore, three dates viz 15th November, 1st and 15th December for seeding and twenty eight wheat genotypes were evaluated in a split plot design with two replications for two consecutive seasons in 2011/12 and 2012/13 at an altitude of 2200 masl of eastern Nepal. The results showed genetic differences and interaction effect of genotypes with the dates of sowing on grain yield, panicle length and effective tillers per square meter. The wheat sown on 1st December showed the highest yield as compared to other sown dates. Similarly, WK1907, WK1911, WK1803, WK1915, WK1909, WK1714 and WK1803 produced highest yield among the tested genotypes with retaining maximum number of effective tillers and posed suitable maturity across all sowing date.

Key words: Genotypes, planting date, wheat, yield

सारांश

गहुँ नेपालको एक प्रमुख खाद्यान्न वाली हो । गहुँका नयाँ प्रचलित जातहरू कम्पै मात्रामा किसानको खेत वारीमा पुगेको पाईन्छ । सामान्यतया, गहुँ बालीको उत्पादकत्व स्थान र जातिय छनौट अनुसार पनि भरपर्ने हुन्छ । तसर्थ, उचित समय र स्थान अनुसारको उपयुक्त जातिय पहिचानको संयोजन बाट गहुँको बढी उत्पादन लिन सक्ने हुन सक्दछ । अझ नेपालका मध्य पहाड जस्ता भौगोलिक अवस्था रहेका स्थानहरूमा उचित समयको पहिचानका साथै गहुँको उपयुक्त जातको समन्वय उत्कृष्ट उत्पादन कालागी एक महत्वपूर्ण जानकारी हुनाका साथै सो बाट उत्पादनको दिगोपनाको सुनिश्चतता प्रदान गर्दछ । यी सबै जानकारी हासिल गर्ने उद्देश्य अनुरूप १५ नोभेम्बर, १ डिसेम्बर र १५ डिसेम्बरको फरक मितिहरूमा २८ गहुँका जातहरू लाई स्पलिट प्लट डिजाईनमा तिन रेप्लिकेशन विधिद्वारा सन् २०१२ र २०१३ मा कृषि अनुसन्धान केन्द्र पाखीबासको २२०० मिटर समुद्र सतहमाथि अवस्थित प्लटहरूमा परीक्षण गरिएको थियो । दुई वर्ष सम्म संचालन भएको परीक्षणले गहुँको जातिय भिन्नता र विभिन्न मितिमा छरिएको प्रभाव बालाको लम्बाई, नलहरूको संख्या र उत्पादनमा आधारित प्रक्षेपण गरि मूल्यांकन गर्दा १ डिसेम्बरमा छरिएको गहुँका २८ जातहरू मध्येमा सबैभन्दा बढी उत्पादकत्व भएकाहरूमा WK1907, WK1911, WK1803, WK1915, WK1909, WK1714 र WK1803 रहेको निष्कौल गरियो ।

INTRODUCTION

Wheat is third most important major staple food crop in Nepal. The wheat crop supplements 20% of food calories contributing food to 36% of the global population. Productions of wheat crop in the country have reached approximately 1883147 mt from 754474 ha of cultivated land with 2.496 t/ha yield (ABPSD 2013/2014). The national statistics shows that the average productivity of wheat in eastern hills was about 2.44 t/ha which is lesser as compared to other hilly regions and national yield (MoAC 2013). In general, wheat grain yield highly depends on water availability, high or low temperatures during the grain filling at maturity and other factors (Gomez-Macpherson and Richards 1995, Andarzian et al 2014). Thus, the identification of sowing date in relation to wheat genotype is an important management option to optimize grain yield potential (Ahmad and Fayyaz-ul-Hassan 2015).

Moreover, cold temperature related sterility is the major reason behind the yield loss where proper adjustment of planting date failed (Sthapit 1988). According to NWRP (2013), a delay of fifteen days from 21st November might reduce the wheat production by 20 to 30%, implying that, identification of the appropriate sowing date to plant wheat genotypes in specific agro-ecological conditions has high implication associated with sharp vertical gradients under mid hill condition of the country. Therefore, the present experiments, as we have aimed possess high importance to find out appropriate sowing dates in association with suitable genotypes in the mid-hill environment.

MATERIALS AND METHODS

Field experiments were laid out during the wheat production seasons in 2011 to 2013 at Agricultural Research Station, Pakhribas, Dhankuta, Nepal located at 2200 masl using split plot design with two replications. Three different seeding dates (15th November, 1st December and 15th December) were used as main-plot and 28 genotypes were placed as sub plot factor. The genotypes comprised of wheat advanced lines from Khumal series, facultative wheat genotypes namely, 18th FAWONN 222 and 18th FAWONN 246, whereas WK1204 was used as popular check variety common to the mid-hill condition of Nepal (NWRP 2013). All the genotypes were obtained from Agriculture Botany Division.

The fertilizers were applied at the rate of 100: 60: 40 kg N: P₂O₅:K₂O (Nitrogen: Phosphorus: Potassium) ha⁻¹ and the seed rate was 120 kg ha⁻¹. All other agronomic practices, e.g. method of planting, seeding rate, planting depth, weeding and harvesting were kept constant for all treatments. The experimental plot consisted of 6 rows of 2 meter long where continuous seeding was done manually. Ten plants from each plot were randomly selected to record the data for plant height and spike length. Similarly, yield was computed by weighing the weight of seeds per spike from four rows of each plot. The major measured traits were number of effective tillers per square meter, maturity days and yield. The number of effective tillers per square meter was calculated by counting tillers during the harvest from one square meter while maturity days were calculated by counting the days between dates of sowing and date to 95% physiological maturity. The plants were harvested and measured separately from different sown date and traits; and computed using M-STAT Software. From the used parameters, mean, coefficient of variation (CV), least significant difference of mean and probability value were calculated.

RESULTS

The levels of significance for different factors and their interactions for various measured traits were presented in Table 2. The analysis of variance showed that the mean squares for genotypes in all dates of sowing were significantly ($P < 0.05$) different for yield (t/ha), number of tillers per square meter and maturity days indicating the existence of genetic variability (Table 2).

Moreover, number of tillers per square meter and yield (t/ha) were significantly ($P < 0.05$) different at 1% level of significance among genotypes over the years. The general trend showed that 10 to 12 average day's diminution for number of days to 95% maturity with every week delayed in planting time (Table 2).

The wheat sown on 1st December yielded 308 tillers per square meter which was the highest among all, followed by sown on 15th November with 295 tillers per square meter. The wheat seeded on 15th December produced the lowest 274 tillers and minimum yield of grain 5.54 t/ha (Table 2), while seeded on 1st December yielded the highest 6.82 t/ha grain. Similarly, crop sown on the first date (15th November) took the highest number of days for maturation, comparing to the wheat sown on other second and third dates.

In comparison among the genotypes, WK1914 produced 394 tillers per square meter followed by WK1902 with 388 tillers per square meter. In addition to this, genotypes WK1905, WK1915, 18th FAWWON222, WK1713, WK1906, WK1901 and WK1907 produced more number of effective tillers per square meter with additional advantage of medium maturity days (Table 2). Among the genotypes, WK1723 was earliest in maturity (161 days) (Table 2). Genotypes WK1714, WK 1902, WK 1803 and WK 1723 produced higher grain yield among all sowing dates with 8.07, 7.66, 7.42 and 7.36 t/ha⁻¹, respectively. Besides them, genotypes WK1907, WK1911, WK1915, WK1909 and WK1712 gave stable yield in all seeding dates.

DISCUSSION

The result showed that the wheat genotypes sown on 1st December had highest yield with highest number of mean tillers per square meter than wheat sown in other dates, in general (Table 2). Our findings showed that wheat sowing can be recommended on around 1st December in high hill localities similar to that of the present experimental site. Similar to the present finding Subedi et al (1985) showed that wheat seeded on 6th December gave maximum yield in mid hill conditions of Nepal. The cumulatively results might imply with increasing altitude probably the wheat should be sown little earlier than approximately in late November, The early (15th November) and delayed (15th December) seeded wheat in present experiments could not exceed the yield of the 1st December. This could be due to yield loss associated with delayed or early sowing inappropriate for the specific mid hill location environmental interaction with the genotypes used. Since the yield is associated with the number of tillers, it has been found that number of tillers per unit area could be affected substantially in early or delayed seeding (Sharma and Garg 2002).

In general, similar results has been obtained elsewhere in late planted wheat because of the problem of field drought that hamper the production of tillers; and thus to the yield (Mohammad et al 2003). The delayed sown wheat produced low number of effective tillers per square meter probably due to drought and rise in temperature, as the plant faces increasingly warm temperature especially towards the maturation period, when plant start to enrich the grains. In present experiment the low number of tillers and yield in delayed sown wheat might be due to warming air temperature at the end of February, when day length inclined to be lengthen supporting rise in air temperature.

The genotypes WK1905, WK1914, WK1902, WK1915, 18th FAWWON222, WK1713 WK1906, WK1901, WK1714, WK1803 and WK1907 had produced more number of effective tillers m⁻² (Table 2). The tiller numbers contributes in one or another way to grain yield (Bassu et al 2010). The higher number of tiller bearing trait could be one of the important characteristics for wheat improvement (Subedi et al 1985), because higher number of tillers favored more adaptation and production comparing to low tiller bearing genotypes. Therefore, tillers per square meter would be the most appropriate traits to be utilized for the wheat improvement.

Among genotypes, WK1714 and WK1803 matured in 145 and 162 days, with mean yield of 8.07 (ton/ha) and 7.42 (ton/ha), respectively (Table 2). The wheat sown on 15th November took higher number of days to mature than comparing to seeded in other dates. This is mainly because of cool temperatures persisted, which resulted in slow seedling emergence and slow subsequent development and fruiting. Similar observation has been mentioned by Subedi et al (1985) that low temperature resulted in slow vegetative and reproductive growth in plants. Since the air temperature is closely associated with the altitude, facing of crop fields to various directions, therefore care should be taken to imply the results of present experiments to be generalized elsewhere. More studies on wheat genotypes yield attribute at various altitudinal gradients in mid hill and southern terai might be of interest for rapid adoption of the wheat farming in applicable localities.

It has been calculated that mean number of days for maturation might be reduce by 12 days comparing to those sown on 15th November and 1st December. An enforced maturity has been noticed to the wheat sown on 15th December (Table 2). Similar observation has also been reported by Sandhu (1993), where duration of reproductive phase such as flowering and maturity were decreased with delaying in

seeding dates. The low yield in wheat sown on 15th November justified that specific timing would be slightly earlier for genotypes used for the specific mid hill localities because of persisting low temperature faced by wheat genotypes around December, January and February which do not stimulate positive attributes for yield performance in the specified locality.

The wheat sown on 1st December were matured in moderate duration comparing to delayed or forced maturity caused either by early or late sowing. This timing of seeding probably perfectly coincided with the yield attributes of genotypes used in specified mid hill localities. Moreover, this timing also perfectly coincided and fit to rice-wheat cropping system in mid hills of Nepal. Therefore, this timing should be recommended for the best yield performance of used genotypes (Table 2) in specific mid hill localities.

Genotypes WK1907, WK1911, WK1915, WK1909, WK1714 and WK1803 produced the highest yield along with at par yield to check variety, WK1204. maturing in medium duration and yielding highest tillers across the planting dates. Therefore, it is likely, these genotypes could be promoted as varieties under eastern mid hill condition of Nepal. Besides that, WK1907, WK1911, WK1915, WK1909, WK1714 and WK1803 were more stable genotypes, which could be utilized under advanced yield and subsequent promotional trials. In addition, based on present findings it is recommendable that the wheat genotypes preferably should be seeded in the first week of December for increased grain production in the eastern hills environments of Nepal.

Table 2. Interaction of wheat genotypes with different dates of sowing and their combined mean for grain yield (t/ha), number of tillers per square meter and maturity days during 2011/2012 and 2012/2013

Genotypes	Yield (t/ha)				Tillers/square meter				Maturity days			
	15 Nov	1 Dec	15 Dec	Mean	15 Nov	1 Dec	15 Dec	Mean	15 Nov	1 Dec	15 Dec	Mean
F-7@-45	6.97	8.22	6.02	7.07	317	255	268	280	176	166	152	165
WK1712	7.25	7.11	5.13	6.50	248	254	235	246	177	165	153	165
WK1713	7.68	7.60	5.30	6.86	316	335	294	315	186	166	149	167
WK1910	7.10	8.43	5.63	7.05	239	246	206	230	182	166	169	172
WK1906	3.07	4.56	2.30	3.31	299	343	291	311	194	185	168	182
18 th FAWWON 222	5.69	6.10	5.27	5.69	414	379	300	364	192	180	169	180
WK1907	4.77	5.88	5.82	5.49	283	342	240	288	199	183	168	183
WK1905	5.74	5.10	5.08	5.31	424	446	411	427	186	181	159	175
WK1976	6.54	5.75	5.03	5.77	272	254	245	257	188	172	149	170
WK1723	7.78	7.96	6.35	7.36	222	288	249	253	168	164	152	161
WK1901	7.66	6.39	5.96	6.67	342	290	282	305	184	168	153	168
WK1909	6.39	6.78	6.15	6.44	256	259	207	241	182	167	156	168
F(5)-1-I-3	6.25	6.08	6.10	6.14	204	256	262	241	182	167	144	164
WK1714	10.1	8.13	5.98	8.07	307	217	205	243	173	169	169	170
WK1904	7.48	6.75	4.92	6.38	300	298	303	300	189	180	145	171
WK1792	5.16	4.92	4.97	5.02	282	312	275	290	172	163	153	163
WK1804	7.64	7.62	6.70	7.32	289	324	234	282	175	172	155	167
WK1204	8.59	8.63	6.41	7.88	268	270	247	262	182	167	156	168
WK1030	4.67	5.99	5.98	5.55	276	291	278	282	186	172	158	172
WK1902	8.84	7.96	6.17	7.66	390	438	336	388	175	174	156	168
WK1914	5.16	6.30	5.35	5.60	396	438	348	394	196	179	168	181
WK1710	6.20	7.01	5.71	6.31	252	276	236	255	182	163	150	165
18 th FAWWON 246	6.25	7.16	4.49	5.97	282	268	228	259	185	169	160	171
WK1915	7.26	7.10	4.81	6.39	336	402	370	369	182	167	163	171
WK1901	7.06	6.65	6.81	6.84	185	260	249	231	182	167	154	168
WK1803	8.28	7.96	6.01	7.42	271	312	363	315	182	168	153	168
WK1481	5.71	6.17	5.93	5.94	258	256	250	255	180	167	153	167
WK1713	7.65	6.60	4.77	6.34	331	303	259	298	182	167	154	168
CV, %	17				21.9				22.3			
LSD (0.05)	1.67				3.2				71.58			
	<0.001				0.14				<0.001			
									0.85			
									0.031			
									2.1			
									7.26			
									9.913			
									0.39			

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Research Note

Effects of Neem (*Azadirachta indic*) and Custard Apple (*Annona reticulata*) Diets on Sterility of House Rat (*Rattus rattus*)

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ABSTRACT

Three different plant products diets – i) neem (*Azadirachta indic* A. Juss) oil mixed diet (neem oil mixed @ 80 ml/kg of normal diet), ii) neem seed powder mixed diet (neem seed powder mixed @ 80 g/kg of normal diet) and iii) custard apple (*Annona reticulata* L.) seed powder mixed diet (custard apple seed powder mixed @ 80 g/kg of normal diet) were separately fed to mature rats (*Rattus rattus*) with single dose feeding of 80 g per pair in a day on 13th week-age during the experimenting years, 2012/013 and 2013/014. In control group only normal diet without neem and custard apple constituents were fed. Sterility test of rat was conducted up to 38 and 28 weeks-age in first and second year, respectively. The test rats were fed normal diet during whole experimenting periods except the one day when they were fed only the neem or custard apple mixed diet on the age of 13th week. Efficacy of the mixed diets on rat-sterility was determined based on pregnancy and parturition by the rats. The two years' results confirmed that all the tested three mixed diets – neem oil mixed diet, neem seed powder mixed diet, and custard apple seed powder mixed diet were effective to stop pregnancy and parturition in rats during whole experimenting periods up to 38 and 28 weeks-age with single dose feeding of 80 g per pair (40 gm/rat) in a day on 13th week-age of the rats; whereas the pregnancy and parturition were observed in the rats that were fed only the normal diet. It is expected, neem and custard apple mixed diets can be utilized in reducing the economically important rodent populations in rice-wheat cropping system in future.

Key words: House rat, rat sterility, neem oil, neem seed powder, custard apple seed powder

सारांश

मुसालाई नपुंसक तुल्याउन तीन थरिका वानस्पतिक आहारा (निमको तेल, निमको बिउको धुलो, सरिफाको बिउको धुलो) मिश्रत दानाहरु छुट्टाछुट्टै खाई मुसाको बाँझोपनमा पर्ने प्रभावकारीताको प्रयोग २०६९/०७० र २०७०/०७१ मा गरिएको थियो। उक्त परीक्षणमा दानाहरु तीन वटा र एउटा वानस्पतिक पदार्थ बिनाको दाना तयार गरी १३ हप्ता पुगेका पाको उमेरका मुसालाई १३ हप्ता उमेर पुगेको दिन ८० ग्राम प्रति जोडी (१ भाले र १ पोथी) का दरले घरमुसालाई प्रयोगशालामा खाईएको थियो। अरु दिन परीक्षण अवधिभर मुसालाई वानस्पतिक पदार्थ मिश्रण नगरिएको दाना खाईएको थियो। परीक्षण कार्य मुसाको उमेर आ. व. २०६९/०७० मा ३८ र २०७०/०७१ मा २८ हप्ता पुग्दा सम्म संचालन गरिएको थियो। मुसाको बाँझोपनको जाँच मुसामा गर्भ रहने तथा सन्तान उत्पादन गर्ने क्षमताको आधारमा गरिएको थियो। दुई वर्षको परीक्षणबाट प्राप्त नतिजाको आधारमा प्रयोग गरिएका तीन वटै (निमको तेल मिश्रत दाना (निमको तेल ८० मि.लि. प्रति किलोग्राम दाना), निमको बिउको धुलो मिश्रत दाना (निमको बिउको धुलो ८० ग्राम प्रति किलोग्राम दाना) र सरिफाको बिउको धुलो मिश्रत दाना (सरिफाको बिउको धुलो ८० ग्राम प्रति किलोग्राम दाना) ले ८० ग्राम प्रति जोडी तथा ४० ग्राम प्रति मुसाका दरले मुसालाई १३ हप्ता पुगेको दिन एकपटक मात्रै खाउँदा परीक्षण अवधिभर मुसामा गर्भ र सन्तान उत्पादन रोक्न प्रभावकारी हुने पाईयो। जबकी वानस्पतिक पदार्थ मिश्रण नगरिएको दाना खाईएका मुसामा नियमित रुपमा गर्भ तथा सन्तान उत्पादन भएको पाईयो। यो प्रविधि भविष्यमा खास गरी धान पछि गहुँ लगाउने खेती प्रणालीमा मुसा नियन्त्रण गर्न उपयोगी हुन सक्ने सम्भावना देखिन्छ।

INTRODUCTION

More than 6000 species of rodents are reported worldwide among which 600 species belonging to genus *Rattus* are called rats (Fall 1977). Thirty species of rodents (rat and mice) are reported in Nepal out of them five are the economically most important common field and domestic rats and mice to damage the agricultural produce in fields and households (Joshi et al, 1991). They are the: house rat (*Rattus rattus*), house mouse (*Mus musculus*), brown rat (*Rattus norvegicus*), lesser bandicoot rat (*Bandicota bengalensis*) and large bandicoot rat (*Bandicota indica*) (Joshi et al 1991). Study on reproductive biology (Shrestha 2001) showed that successful mating of house rat was better in spring (90%) and autumn (90%) as compared to summer (80%) and winter (70%). In those studies, gestation period of house rat was 20-23 days; and male and female were sexually matured on 11th and 13th week after birth, respectively.

Enormous losses is caused by rodents in various ways by - damaging the standing crops and stored products; picking the sown seeds in fields; carrying the food materials in their burrows; spoiling the food grains with their excreta, urine and hair; extensive burrowing the crop fields; damaging the cloths and weaker storage structures; transmitting the rodent born diseases to human and pet animals etc (Joshi et al 1991, Ghosh and Durbey 2003, Lakshminarayanan et al 2015). In India, postharvest loss due to rodents was estimated to be 25-30%. In a 100 x 100 m² godowns, the loss due to rodents could be 4,200 kg estimating that 6 rats can consume the food equal to one man (Ghosh and Durbey 2003).

Rodents mainly the *Rattus rattus*, *Bendicota bengalensis*, and *Mus musculus* are agriculturally more important in Nepal causing the agricultural losses up to 15-25% every year (Shrestha 2001). Shrestha (2001) showed that the movement of house rat in rice and wheat fields in periphery of houses was up to 100 and 150 m, respectively. Yield losses caused by rodents in rice and wheat fields were estimated to be 575 kg/ha and 1132 kg/ha, respectively (Shrestha 2001). The total grain loss in storage by rat was estimated to be 2996 mt per year in Kathmandu valley (Shrestha 2001).

Several methods are available to control rats in fields and households (Joshi et al 1991, Upadhyay et al 1993, ED 1995/96, Mahajan et al 2015). However, in present experiments to minimize the rodent populations in fields and households, we approached method to cause sterility in rats by using botanical-mixed diets. For the purpose series of preliminary works were done in the past (ED 2008, Shrestha 2010, ED 2012). Here, in this paper we elucidate efficacy of some botanical-mixed diets on sterility of house rat.

MATERIALS AND METHODS

Preparation of Test Rats

House rats (*Rattus rattus*) were used as test rats that were collected from the farmers' households. They were kept in cages singly for three weeks in laboratory condition and were reared by feeding the normal diet and water at Entomology Research Division, Khumaltar, Lalitpur, Nepal. After the female rats were recognized not to be pregnant each of them were reared together with a mature male in separate cage for 10 days. The pregnancy of the female rats was checked by pressing the thumb in abdominal cavity. The pregnant rats were reared singly in separate cages until parturition. The newly born young rats were reared together with their mother up to 28 days. The young rats were then separated from their mother and were reared in pair (1 male and 1 female) or in groups until they get matured on 90 days (13 weeks) to be used for sterility test.

Preparation of Normal and Botanical Mixed Diets

Neem (*Azadirachta indica* A. Juss) seeds and Neem oil were collected in July while Custard apple (*Annona reticulata* L.) seeds were collected from August to September from local market. These seeds were separately shade dried and crushed into powder. Normal diet was prepared by mixing the various ingredients at the proportion of 400 g of chicken feed (No: 3), 200 g of whole wheat meal, 200 g of grinded gram, 100 g of broken pieces of groundnut, 60 g of skim milk, 20 g of sodium chloride and 20 ml of mustard oil. The normal diet was used for two purposes - one for maintenance & mass production of experimental rats, and another for control diet to be used as a treatment in sterility test of the rats. The two ingredients - groundnut pieces and mustard oil may not be required for preparing the maintenance and mass production diet.

The normal diet was cooked in boiling water and fed to rats daily for maintenance and mass production. Three different diets - i) neem seed powder mixed diet (neem seed powder mixed @ 80 g per kg of normal diet), ii) custard apple seed powder mixed diet (custard apple seed powder mixed @ 80 g per kg of normal diet), and iii) neem oil mixed diet (neem oil mixed @ 80 ml per kg of normal diet) were prepared for efficacy test on rat-sterility. The four diets (one control/normal and three botanical mixed diets) that were to be used as four treatments in sterility test were prepared in the form of pellets each of 10 g dry weight (11.5 g of wet weight) with the help of pellet making frames. The wet pellets of 11.5 g were oven dried at 60°C for 5 hours or until to dry.

Test of Botanical-Mixed Diets on Sterility of House Rat

Each treatment diet was fed to four pairs (as four replications) of matured rats in a day on 13th week-age with single dose feeding of 80 g (8 pellets each of 10 g) per pair in laboratory/room condition during the experimenting years, 2012/013 (2069/2070) and 2013/014 (2070/2071). Each pair (1 male and 1 female) was kept in four separate cages as four replications - Cage 1, Cage 2, Cage 3 and Cage 4 (Tables 1, 2, 3, and 4). The experiment was conducted starting from 13th week-age and completing on 38th and 28th week-age of the rats, in 1st and 2nd year, respectively. The test rats were fed in normal diet during whole experimenting periods except the one day, when they were fed only the botanical-mixed diet on their 13th week-age. Rat-sterility was determined based on pregnancy and parturition by the rats. Pregnancy was checked by pressing the thumb in their abdominal cavity weekly. Body weight was also observed weekly. Parturition was observed based on gestation period regularly. The diet was supplied to rats at 3-4 PM daily.

RESULTS

During the two years' experiments no pregnancy and parturition was observed in the rats that were fed the neem oil, neem seed and custard apple seed mixed diets. All the tested three mixed diets found effective to stop pregnancy and parturition in rats during whole experimenting periods up to 38 & 28 weeks-age of the rats with single dose feeding of 80 g per pair in a day on 13th week-age of the rats (Tables 1, 2, 3). Whereas, the pregnancy and parturition were observed in the rats that were fed only the normal diets (Table 4). The results of test diets on rat sterility were same in 1st and 2nd year, therefore representative data of only 1st year has been presented here. All the tested mixed diets were effective to stop birth in rats in both years.

DISCUSSION

In preliminary studies (Shrestha 2010, ED 2008) all the three botanical-mixed diets - neem oil @ 20ml/kg of normal diet, neem seed powder @ 100 g/kg of normal diet, and custard apple seed powder @ 100 g/kg of normal diet were effective to stop birth in house rat during whole experimenting periods up to 40 weeks-age when each diet were fed @ 80 g per pair daily for two months in 2004/2005 and three months in 2005/2006. Later in a study (ED 2012) the same botanical-mixed diets that were used in the preliminary works were effective to stop birth in house rat and field rat (*Bendicota bengalensis*) during whole testing period up to 22 weeks-age when each botanical mixed diet were fed @ 80 g per pair daily for two weeks. Present study revealed that all the tested three botanical-mixed diets found effective to cause sterility in house rat during whole testing periods up to 38 and 28 weeks-age with single dose feeding @ 80 g per pair for one day on 13th week-age of the rats.

Table 1. Effect of neem seed powder mixed diet on pregnancy and parturition of house rat

Age in weeks	Cage 1			Cage 2			Cage 3			Cage 4		
	Body wt (g)		Preg-nancy	Body wt (g)		Preg-nancy	Body wt (g)		Preg-nancy	Body wt (g)		Preg-nancy
	Male	Female		Male	Female		Male	Female		Male	Female	
13	100	85	no	90	100	no	140	90	no	130	120	no
14	105	90	no	100	110	no	140	90	no	130	130	no
15	105	100	no	100	115	no	140	90	no	140	130	no
16	110	100	no	105	115	no	150	100	no	130	125	no
17	105	100	no	110	120	no	155	110	no	130	130	no
18	130	120	no	150	130	no	160	130	no	145	150	no
21	150	120	no	150	150	no	170	150	no	150	150	no
24	170	150	no	160	160	no	180	160	no	160	160	no
25	170	160	no	165	160	no	180	165	no	165	160	no
27	180	165	no	175	165	no	190	170	no	180	165	no
30	185	170	no	200	195	no	200	175	no	200	190	no
32	180	165	no	210	190	no	200	185	no	190	180	no
35	190	170	no	220	200	no	220	200	no	210	195	no
37	190	180	no	220	200	no	210	200	no	210	210	no
38	210	190	no	225	200	no	210	200	no	210	210	no

Table 2. Effect of custard apple seed powder mixed diet on pregnancy and parturition of house rat

Age in weeks	Cage 1			Cage 2			Cage 3			Cage 4		
	BW (g)		Preg-nancy	BW (g)		Preg-Nancy	BW (g)		Preg-nancy	BW(g)		Preg-nancy
	Male	Female		Male	Female		Male	Female		Male	Female	
13	190	80	no	13	110	No	100	80	no	120	90	no
14	100	85	no	14	110	No	100	90	no	140	80	no
15	110	110	no	15	120	No	100	110	no	140	100	no
16	120	100	no	16	125	No	120	100	no	135	95	no
17	125	95	no	17	135	No	140	95	no	135	95	no
18	140	120	no	18	140	No	150	120	no	160	110	no
21	150	140	no	21	160	No	170	140	no	170	125	no
24	160	150	no	24	170	No	200	180	no	190	140	no
25	180	160	no	25	180	No	210	170	no	190	150	no
27	190	170	no	27	190	No	220	185	no	200	170	no
30	210	180	no	30	200	No	210	180	no	180	180	no
32	220	180	no	32	200	No	210	185	no	200	190	no
35	220	195	no	35	210	No	210	170	no	200	195	no
37	220	195	no	37	220	No	210	175	no	200	195	no
38	220	190	no	38	210	No	230	180	no	200	200	no

Table 3. Effect of neem oil mixed diet on pregnancy and parturition of house rat (2012/013)

Age in weeks	Cage 1			Cage 2			Cage 3			Cage 4		
	BW(g)		Preg-nancy	BW (g)		Preg-Nancy	BW(g)		Preg-nancy	BW (g)		Preg-nancy
	Male	Female		Male	Female		Male	Female		Male	Female	
13	110	100	no	100	100	No	100	90	no	100	100	no
14	130	135	no	130	110	No	120	110	no	120	110	no
15	145	145	no	140	135	No	155	125	no	135	115	no
16	150	150	no	155	150	No	160	120	no	140	120	no
17	165	150	no	160	150	No	165	135	no	155	135	no
18	165	150	no	165	150	No	170	145	no	160	140	no
21	170	155	no	165	150	No	180	150	no	160	150	no
24	175	155	no	170	155	No	185	155	no	165	145	no
25	175	155	no	170	155	No	190	155	no	170	150	no
27	185	165	no	185	170	No	200	165	no	185	165	no
30	180	170	no	200	170	No	210	175	no	195	170	no
32	200	200	no	230	170	No	210	175	no	230	185	no
35	210	200	no	230	190	No	230	200	no	240	185	no
37	210	190	no	235	195	No	230	200	no	240	195	no
38	210	200	no	240	200	No	240	200	no	250	200	no

Table 4. Effect of normal diet on pregnancy and parturition of house rat

Age in weeks	Cage 1			Cage 2			Cage 3			Cage 4		
	Body wt (g)		Preg- nancy	Body wt (g)		Preg- nancy	Body wt (g)		Preg- nancy	Body wt (g)		Preg- nancy
	Male	Female		Male	Female		Male	Female		Male	Female	
13	110	100	no	100	100	no	110	100	no	100	100	no
15	125	120	no	120	120	yes	125	120	yes	130	130	yes
17	155	145	yes	135	165	yes	155	135	yes	145	165	yes
18	160	160	yes	140	135	par	160	170	yes	155	120	par
19	160	185	yes	155	145	no	160	130	par	165	165	yes
23	165	135	par	160	150	yes	160	185	yes	170	130	par
25	165	170	yes	166	160	yes	175	140	par	190	180	yes
27	175	140	par	175	185	yes	185	170	yes	195	135	par
28	190	145	no	185	140	par	195	130	par	190	140	no
29	220	160	yes	195	150	yes	210	140	no	200	155	no
30	220	175	yes	200	160	yes	220	150	yes	210	165	yes
32	220	195	yes	210	185	yes	220	180	yes	220	180	yes
35	230	170	par	235	160	par	230	155	par	200	195	yes
37	245	175	yes	210	170	no	210	160	no	200	165	par
38	250	180	yes	225	175	yes	215	165	yes	210	175	yes

Rodent damage is very serious to agricultural produce both in fields and households (Upadhayay et al 2093, Lakshminarayanan et al 2015). Mainly the house rat and field rats are more destructive to standing crops/crop produce, stored grains and particularly to rice-wheat cropping system in Nepal. This study has achieved a possible alternative approach to reduce rodent populations and minimize the yield losses in crop fields and households particularly in rice-wheat cropping fields by causing sterility in them. This study confirmed that all the tested three botanical-mixed (7.5% of botanical powder or oil mixed) diets – neem oil mixed diet, neem seed powder mixed diet and custard apple seed powder mixed diet found effective to stop birth in house rat for whole testing periods up to 38 and 28 weeks-age by single dose feeding of 80 g per pair (40 g/rat) in a day on 13th week-age of the rats.

Several studies have shown effect of plant materials which have been successfully used to cause sterility in rats (Upadhayay et al 1993, Lakshminarayanan 2015, Mahajan et al 2015). In addition, this study has provided the clear protocols to prepare the diets for experimental and mass production of rats and preparing of test rats which will be applicable for the future studies. The present study has shown a preliminary experiment causing sterility in rats by single day feeding of the specific ration; however, more detailed physiological evidence based experiments should be performed in near future.

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Technological Advances in Huanglongbing (HLB) or Citrus Greening Disease Management

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ABSTRACT

Huanglongbing (HLB), previously citrus greening disease, is the most destructive of citrus species causing major threat to the world citrus industry. The disease was reported from China in 1919 and now known to occur in more than 40 different countries of Asia, Africa, South and North America. Three species of gram negative bacterium namely *Candidatus Liberibacter asiaticus*, *Candidatus Liberibacter africanus* and *Candidatus Liberibacter americanus* are the casual organisms of HLB, respectively prevailing in the continent of Asia, Africa and South America. It is one of the most extensively researched subjects in citriculture world. HLB was detected in 2004 and 2005, respectively in San Paulo of Brazil and Florida of USA: the two leading citrus production hub of the world causing huge economic loss within 5 years of first detection. Since then research on HLB detection and management was further accelerated in American continents. This paper presents the scientific advancement made on detection, spread, economic losses caused by HLB in different parts of the world and controlling management strategies. Remarkable achievements have been made on HLB detection techniques including iodine test, qPCR and more recently in spectroscopy. While efforts are being made to develop resistance varieties using conventional and biotechnological tools management strategy which includes reduction of inoculums source, vector control and replant with disease-free planting materials still remains major option for HLB control. Citrus intercropping with guava have shown promising results for vector reduction.

Key words: Citrus, citrus greening, huanglongbing, liberibacter, management

सारांश

हवाइलोङ्गबिङ (यच यल वि) रोग, सुन्तलाजात खेतीका लागि संसारभर मुख्य चुनौती हुदै आएको छ। यसको पुरानो नाम सिट्रस ग्रिनडि रोग हो। सन् १९१९ मा सर्वप्रथम चीनबाट यस रोगको बारेमा विवरण प्रकाशित भएको थियो। हाल यो रोग एसिया, अफ्रिका, उत्तर तथा दक्षिण अमेरिकाका ४० भन्दा धेरै देशहरूमा फैलिसकेको छ। एसिया, अफ्रिका र दक्षिण अमेरिकामा क्रमसः क्यान्डिडाटस लिबेरिब्याक्टर एसियाटिकस, क्यान्डिडाटस लिबेरिब्याक्टर अफ्रिकानस र क्यान्डिडाटस लिबेरिब्याक्टर अमेरिकानस प्रजातिका व्याक्टेरियाले यो रोग लगाउछ। ठूलो आर्थिक नोक्सानी गर्ने भएकाले सुन्तलाजात फलफुल बालीमा यस रोगको बारेमा अत्यन्तै धेरै अनुसन्धानहरू भएका छन्। यस बालीको व्यवसायिक उत्पादनका लागि संसारमा नै ठूला मानिएका ब्राजिलको सन्ताउलो र संयुक्त राज्य अमेरिकाको फ्लोरिडा राज्यमा क्रमसः सन् २००४ र २००५ मा यो रोग देखिएको ५ वर्ष भित्रमा धेरै आर्थिक क्षति पुऱ्याए पछि यस रोगको अनुसन्धानले अमेरिकी महादेशमा अझै व्यापकता पायो। यस लेखमा यच यल वि रोगको पहिचान, फैलावट, यसले पुऱ्याउने आर्थिक क्षती र यो रोगको व्यवस्थापनका विषयमा गरिएका बैज्ञानिक प्रगतिका बारेमा चर्चा गरिएको छ। रोग पहिचानको लागि आयोडिन टेस्ट र पिसिआरको प्रयोग हुदै आएकोमा हालका वर्षहरूमा सजिलो र सस्तो स्पेक्ट्रोस्कोपी प्रविधिको प्रयोगमा पनि अनुसन्धान केन्द्रित भएका छन्। रोग नियन्त्रणका लागि परम्परागत र जैविक प्रविधिको प्रयोगबाट रोगअवरोधक जातको विकासका प्रयास भएपनि आशा अनुरूपको प्रतिफल प्राप्त नभएको परिप्रेक्षमा हालसम्म पनि रोगी बोटहरू हटाउने, रोगको कारक व्याक्टेरियाको संवाहक सिट्रस सिल्ला किरा नियन्त्रण गर्ने र निरोगी कलमी विरुवाको प्रयोग गरी पुनरोपण गर्नु नै यस रोगको व्यवस्थापनको विधि रहेको छ। यच एल बी रोगका सम्बाहक नियन्त्रणको लागि सुन्तला र अम्बा संगसंगै लगाउदा केहि आशाजनक नतिजा प्राप्त भएको छ।

INTRODUCTION

Globally, citrus fruit represents one of the most important commodities with respect to production and trade. In production volume, it occupies second position after banana and plantain (Statista 2012) and ranks top position in international trade in terms of value (UNCTAD 2011). In Nepal, citrus especially mandarin, sweet orange and lime, are among the commodities having high potential for commercial production. The mid-hill region of Nepal have favorable conditions for commercial production of citrus because of agro-climatic suitability, access to domestic and regional markets and past experiences of farmers on citrus farming. As a result of these reasons along with government priority for its commercialization the area under citrus cultivation and its total production have increased substantially over the years. In the year 2003/04, 148,010 mt of fresh citrus fruit was produced in Nepal from 24,799 ha area. The total area under citrus cultivation has increased to 36,975 ha and total production to 216,188 mt in 2012. However, productivity of citrus decreased to 9.14 mt/ha in 2012 compared to 10.62 mt/ha in 2003 ((MoAD 2013).

Among many biotic factors contributing to low productivity and threat to citrus industry huanglongbing or greening disease, hereafter HLB has been considered the number one in Nepal (Regmi and Yadav 2007, Roistacher 1996) and the world (Bové 2006, 2014). HLB was first reported from southern China in 1919 and is now known to occur in more than 40 different Asian, African, Oceanian, South and North American countries (Bové 2006).

In Nepal, the disease was first reported from Pokhara in 1968 (Thrower 1968) as the main cause of citrus decline in the country (Roistacher 1996, Regmi and Yadav 2007). The disease is caused by a phloem-restricted, non cultured, Gram-negative bacterium transmitted by citrus psyllid insect vector; non-curable once trees are affected. Since HLB is very destructive disease and slowly invading new citrus growing areas represent a major threat to the world citrus industry including Nepal.

Mandarin (Suntala), sweet orange (Junar) and acid lime (Kagati) are the major commercial citrus species grown in Nepal (MoAD 2013). Since all citrus species are infected with HLB in varying degree and the disease is already spread several parts of the country (Bove 2006) understanding the complex nature of the disease and its management approaches is very vital to save the citrus industry of the country. In this context recent updates on technological information on detection and control management of HLB would be worth to be elucidated. Therefore, this paper presents a review on history, geographical distribution, detection and management of HLB.

CAUSAL ORGANISM

Citrus greening disease or HLB was first reported from China in 1919 by Reinking while evaluating diseases of economic plants in southern China and used English term "yellow shoot" of citrus in the report, however, for a long period it (at that time the name "HLB" was not used) was thought to be present in China (Bove 2006). At that time it was believed that the HLB was caused by abiotic factors like Zn deficiency/toxicity and poor drainage system (Oberholzer et al 1965). By 1967, it became established that greening was graft and insect transmissible with conclusion caused by virus (Bove 2006). In 1967, mycoplasma like organisms (MLOs) were believed to be associated with plant diseases mostly with "yellow" symptoms resembling with greening symptoms. On close examination, these organisms were seen to have bacterial cell wall in addition to cytoplasmic membrane, suggesting that they were gram negative true bacteria (Garnier and Bove 1977). Thus, it was concluded that the HLB agent was gram negative bacterium. Later on it was confirmed by Electron Microscopy that South African "greening", Indian "dieback", Taiwan "likubin" and Philippines "mottle leaf" all were caused by HLB bacterium (Bove 2006).

Using universal primers, the 16s ribosomal DNA of two strains of the bacterium obtained by Polymerase Chain Reaction and comparisons with gene sequences showed the HLB organism belonged to the subdivision of the class Protobacteria, but were distinct from other members. The bacterium from Asia named *Candidatus Liberibacter asiaticus* (Las), and the African *Ca. L. africanus* (Laf) (Garnier et al 2000). *Ca. L. americanus* (Lam) is the new species identified in 2005 causing HLB in Brazil (Teixeira et al 2005).

The three species of HLB causing bacteria differ in their pathogenicity due to combination of environmental conditions and insect vectors (Jagoueix et al 1996). The African strain of HLB is heat sensitive and does not cause symptoms at temperatures greater than 25–30° C. The Asian strain, primarily distributed in Asia, is heat tolerant and able to cause symptoms at temperatures greater than 30° C. The American strain, which is reported from Brazil and Mexico, appears to have heat tolerance similar to that of the African strain (NAPPO 2012).

HISTORY

Origin of HLB

Although HLB was first reported from China where many citrus species were originated most scientists believe that citrus species are not the original host of the HLB organism (Beattie et al., 2005, Bove, 2006 and Graca, 2011). There are different opinions among researchers about the origin of HLB. One of the hypothesis is that Asian HLB originated in citrus in India from unidentified native rutaceae and may have moved to other Asian countries through planting materials and citrus psyllids including China and other south-east Asian countries (Graca 2011).

Beattie et al. (2005) have proposed another hypothesis that the disease may actually originated in Africa, possibly in an asymptomatic host such as *Verpris lanceolata*; then transmitted to citrus by insect in European settlement on the east coast of Africa and then taken to the Indian subcontinent in infected plants or budwood 300 to 500 years ago, and then into China later. It has been recently suggested that the liberibacters have Gondwanan origins (Beattie et al 2005, Bove 2006).

At present HLB has spread to most of the citrus growing countries of the world. Only the areas free from HLB as well as of the psyllid vectors of the disease are Mediterranean basin, most of Western Asia (Near and Middle East), Australia, and North- and South-Pacific islands are still free of HLB (Bove 2006). Detail updated information of the disease distribution can be obtained from the website: <http://www.cabi.org/isc/datasheet/16567>.

China

Reinking (1919) used English word "yellow shoot" of citrus, a disease thought to be of little importance in those days. Later surveys showed that by 1936 the disease had spread to become a serious problem. The most extensive work on HLB in southern China was conducted from 1941 to 1955 by Lin Kung Hsiang (Bove 2006). From the discussions with the farmers of the Chaozhou county in Guangdong province, it was learned that HLB actually had been there since the 1870s. Based on this Lin postulated that HLB originated from Guangdong Province, China. He conducted several exploration in China and disclosed that farmers of Chaozhou district had given the name "huang long bing" to the disease, where "huang" means yellow, 'long' meant to 'dragon' and "bing" stands for disease. Hence, the disease was called "yellow dragon disease". The most outstanding result obtained by Lin was the demonstration, by precise experimental work, that HLB is a graft-transmissible, infectious disease, and should neither be attributed to physiological disorders such as mineral deficiencies or water logging, nor to soil-borne diseases such as nematode infestation or *Fusarium* infection (Bove 2006). For these reasons, the International Organization of Citrus Virologists (IOCV) accepted the official name of the disease be huanglongbing (HLB) in 1995 at the 13th conference of the Organization in Fuzhou (Fujian, China).

Africa

A disease similar to HLB, was observed in South Africa in 1928 under the name "greening". However, the true nature of the disease was not immediately recognized, and in the first description of "greening" in 1937, the problem was still assumed to be mineral toxicity (Oberholzer et al 1965). Through a collaborative research by A.P.D. McClean (phytopathologist) and P.C.J. Oberholzer (horticulturist)

demonstrated that greening was transmissible by graft inoculation as well as by the African citrus psylla, *T. erytrae*. The infectious nature of “greening” was thus established. The name “greening” was adopted by the scientific literature until “huanglongbing” was given as the official name in 1995, probably because of the proximity of western scientists to South Africa (Gottwald et al 2007).

Philippines

The disease became a serious problem in the late 1950s was described in 1921 with the name - “mottle leaf” and thought to be related to zinc deficiency, because the symptoms of “mottle leaf” were very similar to those of HLB in China and Taiwan, and “greening” in South Africa (Salibe and Cortez 1968). Being graft-transmissible, HLB has also insect vectors: the African citrus psyllid, *Trioza erytrae*, reported as the vector in South Africa in 1965 and the Asian citrus psyllid, *Diaphorina citri*, identified as the vector in Asia (India and Philippines) in 1967 (Bové 2006).

Other South-east Asian Countries

In Indonesia, HLB became a major problem identified first time in the name of “Vein phloem degeneration” or “phloem necrosis” in 1940s (Tirtawidjaja et al 1965). HLB first appeared in Thailand in the 1960s and was so severe that the length of time between the onset of the disease and debilitation of the entire tree was only about two years (Schwarz et al 1973). HLB disease, locally called “Likubin” was first identified in Taiwan in about 1930, but not considered as serious problem until 1957 (Gottwald 2007). The HLB inoculums was assumed to be brought into Taiwan from southern China through some infected citrus scions or seedlings and thought to be associated with poor soil conditions such as deficiency in essential nutrients or poor drainage (Su 2008). Other south Asian countries where HLB has also been detected by DNA hybridization and/or PCR include Myanmar, Malaysia, Cambodia, Laos and Vietnam (Bové 2006).

India

Citrus in India has been known to suffer seriously from certain disorders resulting in low production, twig dieback, slow death and even sudden wilting attributed to “dieback”, a disease that was first observed in the 18th Century in central India (Bové 2006). Proof for the presence of HLB in India was eventually obtained at the virus Research Center at Poona by Capoor and co-workers, when they succeeded in transmitting the HLB pathogen by the Asian psylla, *D. citri* by demonstrating that trees with dieback symptoms invariably proved positive for HLB (Capoor et al 1967). Thereafter, it was reported from different citrus growing regions of India and was considered to be principal cause of citrus dieback disease (Das 2008). From several surveys conducted from 2007 to 2012 along with molecular test (real-time PCR) in 16 states of India confirmed its distribution in all studied states (except Arunachal Pradesh): Andhra Pradesh, Assam, Karnataka, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Punjab, Rajasthan, Sikkim, Tamil Nadu, Tripura and West Bengal (Das et al 2014).

Pakistan

Several surveys conducted during 1991 showed that citrus greening disease (CGD) is one of the most important and severe disease in Punjab and North West Frontier Province having an incidence rate of 22% in Kinnow, 25–40% in sweet orange, 15% in grapefruit, 10% in sweet lime, and 2% in lemon (Batool et al 2007).

Nepal

Citrus decline was reported for the first time in Pokhara valley by Thrower (1968). Based on visual observation, Knorr et al (1970) suspected that the decline was caused by greening disease entered with the planting materials introduced to Horticulture Research Station, Pokhara from Saharanpur, India. About 55% of citrus trees in Pokhara valley and 100% in Horticulture Research Station were symptomatic to HLB in 1980s (Regmi 1982). Later more surveys and studies were carried out in other parts of the country to explore the distribution of greening disease and its vector (Regmi and Lama 1988, Regmi 1994, Regmi et al 1996). More recent PCR test showed that HLB is widespread in many citrus pockets of Kaski, Syanja, Tanahu, Lamjung and Dhading districts (Bové 2006, Regmi and Yadav 2007, Regmi et al 2010).

America

Brazil has been the first American countries to report HLB, in March 2004 San Paulo State eliminated about 6 million infected trees by 2008, (Lopes et al 2011). The major HLB agent was the new liberibacter species *Ca. L. americanus*, present in 92% of the trees (Bové 2006). From 2005 on, the rest of America and the Caribbean islands started to be invaded by Las and *D. citri*: first Florida in 2005, then Cuba in 2006, followed by Mexico, Belize, Central America in 2009, Argentina, California, Guadeloupe and Texas (Bové 2014).

DETECTION OF HUANGLONGBING

Visual Symptoms

Citrus trees can be suspected to be affected with HLB by visual symptoms appeared on leaves and fruits (Bové 2006). A tree infected with HLB in the field usually develops one or more yellow shoots with other parts of the tree healthy or symptomless. The affected leaves develop a pattern of yellow and green areas lacking clear limits between the colors, giving a “blotchy mottle” appearance. This is the most characteristics foliar symptom and the patterns are asymmetrical on the two halves of the leaf (Bové 2006). Leaves can also become thicker, with veins enlarged and corky in appearance.

In later stages, Zn deficiency-like symptoms can be seen followed by leaf drop and twig dieback (Gottwald et al 2007). Symptomatic fruit are small, lopsided and as they mature and ripen the stylar end remains green, hence named as "greening" (Bove 2006). When cut in half, small, dark aborted seed can be observed and vascular bundles in the fruit axis are discolored. Fruit, especially sweet oranges, can also have mottle appearance and if the peel is pressed with finger, a silvery of the depressed area will result (Bove 2006). Visual symptoms of HLB noticed on leaves and fruits are varied and can be resemble other disorders like micronutrient deficiencies, such as zinc, manganese and iron (Etteberria 2007).

Johnson et al (2014) found that greening causes a loss of 30-50% of trees' fibrous roots before symptoms are visible above ground. HLB bacteria enter trees through leaves but the disease attacks roots long before the leaves show signs of damage. Once bacteria enters through psyllid sucks into leaves, the bacteria travel quickly to the roots, where they replicate, damage the root system and spread to the rest of the host tree's canopy (Johnson et al 2014).

Molecular Diagnosis

For the diagnosis of HLB, two molecular techniques have been used: conventional Polymerase Chain Reaction (PCR) and real time PCR (RT-PCR), also known as quantitative PCR (qPCR) that are based on the use of PCR primers that amplify DNA sequences of the Liberibacters associated with HLB (Bove 2006). Conventional PCR methods use specific primers that amplify the sequences of the rDNA 16s genes and primers based on the proteinaceous genes (operon-B) (Jagoueix et al 1996, Tian et al 1996, Hocquellet et al 1999, Teixeira et al 2005). The low concentration and irregular distribution of the pathogen in host plants, along with the inhibitors of PCR present in citrus extracts made the detection of the pathogen difficult. Although conventional PCR and qPCR are accepted techniques for the confirmation of trees symptomatic for HLB in Brazil and the United States, qPCR is considered much more sensitive and robust than conventional PCR and the technique has been validated with DNA extracts from different species of citrus and different tissues from diverse geographic regions (Li et al 2006, 2007).

Biological Indexing

Although qPCR is currently the method of choice for diagnosis of HLB (Li et al 2006), biological indexing techniques are also available (Taba et al 2006). Due to low rate of graft transmission of the bacterium associated with HLB, the success rate for biological indexing of HLB is variable. The appropriate indicator plants are sweet orange or *Orlando tangelo* for African HLB and sweet orange or Ponkan mandarin for Asian HLB (Roistacher 1996). It is known that presence of the citrus tristeza virus can interfere with HLB symptom expression; and if CTV is present, grapefruit may be used as an indicator (Roistacher 1991). The preferred inoculation technique is the side graft, with leaf grafts being the alternative. The seedling indicators are trained to single leaders and held at 20 – 25⁰ C for African HLB and 25 – 32⁰ C for Asian HLB. Symptom expression is the typical mottle and chlorosis. The shoots are distinctly smaller, more chlorotic, and with smaller leaves than the uninoculated controls. Symptoms should appear 8 to 12 weeks after inoculation. Further details can be obtained in Roistacher (1991, 1996).

Iodine Test

Although the PCR test for HLB is reliable, it is considered highly expensive and labor demanding thus not suitable for indexing large numbers of samples (Etteberria 2007). To resolve this problem, a rapid, simple field diagnostic iodine test that could be used to prescreen samples intended for PCR analysis has been developed (Taba et al 2006). Anatomical studies conducted in the 1960s, showed "massive accumulation" of starch in leaf samples from HLB infected sweet orange trees (Etteberria et al 2009). The accumulation of starch in HLB infected leaves has been found up to six times more than healthy leaves. Since the starch readily reacts with iodine, resulting in a very dark-grey to black stain, thus this technique has been adapted as one of the easiest diagnostic tool for HLB. These diagnosis methods have been reported to be more than 90% of agreement between PCR analysis and starch tests with iodine (Etteberria 2007). Iodine solutions products labeled as either "tincture of iodine" or "iodine tincture" in 1 to 10 with water can be used.

Takushi et al (2007) also showed scratch method for HLB detection in citrus leaves using iodine-starch reaction. They found average quantity of starch was 514.2 mg/kg in HLB infected leaves and 85.6 mg/kg in healthy leaves (Welch's *t*-test $p < 0.01\%$), a significant difference in quantity of starch between disease and healthy leaves. Based on this result, they devised Scratch method that uses sandpaper for HLB diagnosis. Accuracy of the scratch method diagnosis in field showed more than 90% of agreement rates to PCR assay. Chamberlain and Irely (2008) compared starch-based field test for HLB to the results from real-time PCR for testing of field samples from 1759 suspected symptomatic trees. They noted that 85% of the samples were positive by RT-PCR versus 78% positive for the starch test. Therefore, they recommended that the starch test should be considered a useful tool for HLB diagnosis in the field, but not as a substitute for PCR-based testing.

Spectroscopy

In recent years, especially after detection of HLB in Brazil and Florida, USA several studies have been initiated to develop more efficient and cost-effective HLB detection techniques using spectroscopy. In a feasibility evaluation Sankaran et al (2011) found about 92% accuracy when they applied visible-near infrared spectroscopy technique for field detection of HLB in citrus trees using spectral reflectance data from the wavelength range of 350–2500 nm.

Hawkins et al (2010) found fourier transform infrared–attenuated total reflection (FT-IR-ATR) spectroscopy as a potentially suitable and inexpensive technique for rapid and early detection of HLB. The mid-infrared region of the spectrum reveals dramatic changes that take place in the infected leaves when compared to healthy non-infected leaves. The carbohydrates that give rise to peaks in the 900–1180 cm^{-1} range are reliable in distinguishing leaves from infected plants versus non-infected plants. Furthermore, airborne

multispectral and hyperspectral imaging technology has also been tested recently for rapid detection of potentially diseased trees over a large area using hyperspectral imaging software (ENVI, ITT VIS) for data analysis with 87% accuracy (Kumar et al 2012).

Manjunath et al (2015) tested a unit Smart-DART™ connected to an Android device to detect *Liberibacter* in psyllids which is portable, affordable, sensitive and rapid to test. According to them with this device Psyllids can be tested to assess the prevalence of *Liberibacter asiaticus* in a population and for early detection of HLB in new areas being invaded by the psyllid.

In Barazil, Jarbas Caiado de Castro Neto, and his research team, spent one and a half years developing an inexpensive and portable way to detect citrus greening before the trees exhibit visible symptoms (Freshplaza, 2014). As mentioned in this report the end product is about the size of a credit card scanning machine, contains a laser, a spectrometer, and a processor to analyze the results. The principle behind this technique is that the diseased and healthy orange tree leaves emit different fluorescence spectra. Leaves affected by HLB emit more light in the middle of the visible spectrum, but have a lower peak - around 750 nm associated with the degradation of chlorophyll in the leaves. The spectrographic signature of citrus greening disease also appears almost immediately after a tree is infected, compared to the year or more it takes for visible symptoms to develop and this portable spectroscopic system called Agricultural Optronics Systems (AgriOS), is reported to detect citrus greening with greater than 95% accuracy (Freshplaza 2014).

ECONOMIC LOSS

It is well known that HLB is one of the most serious citrus diseases worldwide, because once a tree is infected there is no cure and its yield is greatly reduced while the disease spreads rapidly. When, HLB becomes endemic, the disease progresses throughout the tree canopy and in the orchard very fast thereby reduce the economic life of affected orchards (Bove 2014). In many countries where HLB is present, it becomes a limiting factor for citrus production. It has been difficult to convince citrus farmers and regulatory agencies to take part in HLB management programs, because HLB management is based on elimination of diseased trees and reduction of vector population demands continuous efforts which is a costly undertaking (Bassanezi and Bassanezi 2008). Economic losses caused by HLB have been estimated in many countries and literatures on the importance and impact of the disease are available. Models that describe the disease incidence progress, disease severity progress and disease severity-fruit yield relationship according to the age of trees are also developed (Stuchi et al 2011, Roistacher 1996). In younger trees, the disease incidence and severity progress is faster than in older ones (Lopes et al 2011). Bassanezi et al (2011) have developed a mathematical model to describe HLB severity and yield relationship. According to this model HLB severity (x) of a grove allows prediction of the expected yield (y) using the formula: $y = \exp(-1.8x)$, $r = 0.33$.

Since the 1960s, Thailand has been plagued with HLB and has had a large impact on Thailand's citrus production. Under the presence of HLB, at the end of 8 years, a farmer would be losing \$1,482 per acre, whereas if the grove was able to survive to year 10, a profit of approximately \$1,370 per acre could be realized (Roistacher 1996). According to him in Thailand, profit is not realized unless trees survive to reach 10 years, as it takes time to recover the costs of planting and caring for the tree until production revenue is high enough to cover costs. The key problem was that in some regions of the country, groves were only lasting 6 to 8 years leading to 10 to 15% tangerine trees destruction each year. Indonesia was severely affected by HLB between 1960 and 1970 with an estimated loss of 3 million adult trees and much higher number of younger nursery plants (Gottwald et al 2007). By early 1960's the Philippines citrus plantation were estimated to cover 25,000 hectares. Ten years later 5 million trees were affected by HLB and the area planted to citrus was reduced to 60% (Aubert 1992).

The economic impact of HLB in Florida has also been assessed. HLB was first detected in Florida in 2005 and by 2014 this disease has caused significant economic and production losses costing additional US\$ 750/acre for HLB management (FreshPlaza 2014). Hodges and Spreen (2012) estimated that a reduction of 23% in orange production during 2006 and 2010 was attributed to the presence of HLB. As a consequence, Florida's economy suffered a \$3.9 billion loss resulting to total output, total value added and labor income decrease.

In Paraguay, HLB was first detected in March 2013. To prevent the spread of disease about 600,000 adult citrus plants and approximately 2,500,000 citrus saplings belonging to some 320 nursery owners were destroyed within two years in Paraguay costing about one million US\$ to government as compensation to farmers for destroyed trees (FreshPlaza 2014a).

Quality of fruit juice is also affected by HLB. Goodrich-Schneider et al. (2008) analysed sweet orange juice hedonically for overall acceptability of flavor and sweetness among consumer panels ($n=100$). The juice from greening-affected fruit was always significantly less acceptable ($P<0.05$) than juice from healthy (control) fruits. Juice from greening-affected fruit was rated as significantly less sweet and significantly lower in orange flavor than juice from control fruit for all panels ($P<0.05$). Juice expressed from visually normal fruit from HLB affected trees generally fell between control juice and greening-affected fruit juice in terms of overall acceptability, sweetness and orange flavor.

HOST RANGE

Huanglongbing is a disease of rutaceous plants (Bove 2014). None of the citrus fruit species are resistant to HLB (Bove 2006). However, responses of citrus species to '*Ca. L. asiaticus*' has been found variable. It severely affects sweet orange, mandarin and tangelo trees but many other species show more or less pronounced symptoms of the disease. Mexican lime (*Citrus aurantifolia*) is less susceptible than sweet orange and mandarin even though it is a preferred host of the vector *Diaphorina citri* (Roistacher 1996). Based on severity of HLB symptoms and the ability to continue growth of the plants inoculation with '*Ca. L. asiaticus*' Folimonova et al (2009) grouped citrus genotypes into four categories as (i) sensitive: *C. halimii*, Nules clementine mandarin, Minneola tangelo, sweet oranges and grapefruit (ii) moderately tolerant: Sun Chu Sha mandarin, sour orange, volkamer lemon, *C. macrophylla*, wingle citrumelo, citron, Palestine sweet lime, acid lime, calamondin, and *C. micrantha* (iii) tolerant: Eureka lemon, Persian lime, Carrizo citrange, and *Severinia buxifolia* (iv) variable (some branch sensitive and some branch tolerant): pummelos, *C. amblycarpa*, cleopatra mandarin, *C. indica*, and

meiwa kumquat. Ahmad et al. (2011) also classified citrus species into very susceptible, susceptible and tolerant categories based on reaction with HLB pathogen.

MANAGEMENT STRATEGIES

Inoculum Reduction and Vector Control

Currently, there is no established cure for this century-old emerging disease. There is general consensus throughout the world on three general management practices for the management of the disease; the planting of certified clean planting materials, effective control of its vector psyllid populations and removal of infected trees that serve as an inoculum source for psyllid acquisition (Bove 2006). These management practices must be adopted in order to have a successful citrus greening management program (Stuchi et al 2011). However, HLB management is difficult, if inoculum sources are widespread and the psyllid vector is well established.

There are no HLB tolerant commercial citrus species and cultivars at present to replace declining healthy trees (Bove 2006). The general control strategy has been to eradicate all existing sources of HLB within an area, then replant with HLB-free trees grown from clean budwood. Psyllid populations must also be reduced as much as possible. Biological control of the psyllid vector is only possible in locations that do not favour build-up of psyllid populations and is often compromised when hyper-parasites are present.

Preventing HLB from entering to healthy area is much easier than trying to eradicate or control it. It is important to avoid bringing propagation materials from HLB-infected areas to non infected area (Abdullah 2009). Zhang et al. (2011) has described various situations on which removal of infected trees and replanting is feasible. One strategy is to immediately reset trees that were removed. However, since psyllids are attracted to new growth and young trees flush more frequently than immature trees, young trees in a block with mature trees supporting psyllids are at greater risk than solid set young trees in a newly-planted block. Pruning may also be an extremely hazardous practice in areas under high HLB pressure, if a careful vector control program is not applied (Stuchi et al 2011) because pruning enhance new flush growth which harbor psyllids. Another strategy is to continue to remove infected trees until reduced tree numbers make the block economically unproductive, then the remove remaining trees and the entire block is replanted (Zhang et al., 2011).

Stuchi and Girardi (2011) have given detail information on various factors associated with the success or failure of HLB management based on inoculum reduction and control of psyllid vector populations by insecticide treatments adopted in Brazil. According to them HLB management practices adopted in Brazil are (i) selection of naturally occurring tolerant materials (ii) new regions for citrus production (iii) screen-house nursery trees production (iv) use of repellent and attractive plants for vector (v) protected cultivation (vi) intercropping and ultra high density (UHD) plantings (vii) intensive production systems (viii) the use of larger nursery trees.

Chemical Control

Zhang et al (2011) reported that the combination of penicillin and streptomycin (PS) was effective in eliminating or suppressing the Las bacterium and provided a therapeutically effective level of control for a much longer period of time than when administering either antibiotic separately using a propagation test system with Las-infected periwinkle and citrus plants. However, Bove (2006) suggested that antibiotic treatment has only short term effect and therefore, is not a sustainable approach in HLB control.

Nutrition

The use of nutritional applications to control or offset the deleterious effects of HLB has been a topic of considerable discussion and debate (Spann et al 2011). However, most reports are anecdotal and without sufficient statistical validity. To develop empirical evidences Gottwald et al (2011) compared combinations of nutritional components consisted of phosphite with Mn-carbonate, Mn-metalosate, Cu-metalosate, or Zn-metalosate, and injection treatments using soluble copper or silver mixed with a polymer along with a control consisting of a standard insecticide program for psyllids. There was no significant difference in titer dynamics, yield (number fruit per tree, kg fruit/tree, proportion of fruit dropped), or quality (Brix, acid, brix:acid ratio), compared to the control. The additional nutrition did not sustain tree health, yield, or fruit quality. Rather, there is a major concern that nutritional supplements may promote area-wide inoculum buildup and spread (Gottwald et al 2011).

Practice of adding additional nutrition, especially the micronutrients expecting extended productive life of infected trees has been a common practice in China (Spann et al 2011). But review of 60+ years' history of research and field practice in China reveals no consistent evidence to support the notion that nutrient management can maintain productivity of HLB-infested trees over the long term (Xia and Sequeira, 2011). Relationship of plant nutrition and HLB infection has been studied in four commercial citrus groves in Florida (Spann et al 2011). Preliminary results of the research showed that HLB-infected trees are consistently deficient in Ca, Mg, Mn, Zn and B, and in an orchard, these nutrient deficient trees are more likely to be HLB-infected than the nutrient sufficient trees. The researchers are not yet clear whether these deficiencies were a result of decreased nutrient uptake, or if the nutrients were being bound within the plant making them unavailable. HLB-affected trees characteristically suffer from a damaged root system and nutritional disorder because of interrupted transport of photo-assimilates from shoot to the root, and mineral nutrients/water from root to the shoot (Spann et al 2011).

He et al (2011) conducted greenhouse experiments to examine the effects of growth-priming agents including root growth enhancer, trace element combination (Zn, Cu, and Mn), plant growth regulators, and sugar transporter alone or in combination on HLB-affected citrus seedlings. When applied alone, root growth enhancer was most effective in promoting plant growth followed by trace element combination as indicated by a significant increase in shoot and root biomass, particularly root length and surface area, as compared with the control (He et al 2011). The main cause of visible HLB symptoms, yield reduction, and tree decline appears to be disruption of phloem tissue, which blocks the flow of photosynthate and nutrients from source to sink tissue (Bove 2006). If supplemental nutrition is

a sustainable approach, it is expected that foliar nutrients will reduce or eliminate damage and plugging in citrus phloem tissue caused by the bacterium and possibly reduce spread or replication of the bacterium in infected trees (He et al 2011).

Use of Tolerant Rootstocks

In Brazil, 12 rootstocks namely two Rangpur limes (FCAV and Limeira), three trifoliate oranges [Rubidoux, FCAV, and Flying Dragon (FD)], *Swingle citrumelo*, Sunki, and Sun Chu Sha Kat mandarins, *Orlando tangelo*, *Carrizo citrange*, and the hybrids Changsha × English Small and Rangpur lime × *Swingle citrumelo* have been tested for HLB reaction (Stuchi et al, 2011). After a first 7-year evaluation cycle for HLB infection the average cumulative incidence (CI) of HLB was 72.1%, with CI values varying from 10 to 100%. For most of the rootstocks, CI values were equal or higher than 60%, while the trees budded onto the FD rootstock showed the lower HLB incidence (10%). Albrecht and Bowman (2012) used greenhouse inoculation tests and field trials to investigate the tolerance of some *Citrus* × *Poncirus* hybrids to infection of *Candidatus Liberibacter asiaticus* (Las). The citrus rootstock US-897 (*Citrus reticulata* Blanco × *Poncirus trifoliata* L. Raf.) was observed to be tolerant to HLB in field plantings (Albrecht and Bowman 2012).

Guava Intercropping

Since long, farmers from Mekong Delta of South Vietnam have been practicing interplanting of citrus with guava and those citrus orchards planted with guava showed much lower psyllid infestation levels and low incidence of HLB compared to citrus orchards lacking guava (Hall et al 2007). This practice adopted by Vietnamese farmers drew the attention of scientific communities of many countries during 1990s and various scientific studies were initiated to understand the scientific reasons. The first of this kind of research was a collaborative research projects involving Vietnamese, Japanese and Australian scientists (Beattie et al 2006). The findings of this research which was presented in a meeting held during December 2006 in Japanese International Research Center showed that interplanting citrus with guava negated infestations of Asian citrus psyllid and consequently HLB (Beattie et al 2006). This study collected the scientific information that young citrus interplanted with guava remained disease-free for a year whereas sole citrus crop showed signs of the disease within four months of planting and reached over 30% trees infected within a year (Hall et al 2007).

A team of American scientists who also made a observation tour to Vietnam in April 2000 noted that the normal life of sole citrus plantings in Mekong region of Vietnam was 2 to 4 years, but those interplanted with white guava were surviving for up to 15 years (Gottwald et al 2010). Although raising guava as an intercrop reduced psyllid population in citrus orchards (Hall et al 2007), the mechanism by which this occurs was unknown. It was postulated that the effects of guava on citrus psylla could be due to mechanical/physical disruption on host recognition, repellent effect of volatile compounds from guava or chemical alteration of the volatile compounds emitted by citrus reacting with guava compounds. From various studies conducted in Florida, USA to determine, if guava has a repellent effect on adults of Asiatic citrus psyllids. Gottwald et al. (2010) noted high adult mortality rates occurred when psyllids were confined to guava in no-choice situations, with 95% mortality occurring within 6-9 days. They postulated that the effect may be due to volatile compounds produced by guava that are deleterious to psyllids. In another study, Zaka et al (2010) evaluated the repellent effect of guava leaf and factors attributed to this activity, response of adult psyllids to guava leaves and its odor in cage test and Y-tube olfactometer test. The olfactometer response of adult psyllids to guava leaf odor was dose-dependent and both male and female psyllids responded similarly to the guava leaf odor.

Since more than 75% of the Florida citrus crops has been infected with HLB, USDA has provided 43 million US \$ for its research (FreshPlaza 2015b). Funded projects include a grant to the University of Florida to develop a bactericide that can be applied to infected citrus trees to reduce or eliminate pathogens, a project at Kansas State University to develop a therapeutic delivery system that will prevent *Candidatus Liberibacter asiaticus* from infecting plants or prevent the development of HLB in infected citrus, and a grant at the University of California-Davis that focuses on using new genetic approaches to managing the Asian citrus psyllid that causes HLB (FreshPlaza 2015b).

CONCLUSION

Review of scientific literatures revealed that HLB is the most devastating disease of citrus fruit crops. Most commercial citrus species of Nepal like mandarin and sweet orange are very susceptible to the disease while acid lime is slightly tolerant, but it is carrier of HLB bacterium serving as hidden source of inoculum. The disease is present in Nepal since early 1960s and has spread many commercial citrus pockets of the country over the years (Bove, 2006 & 2014, Regmi et al, 1996 & 2010, Regmi and Yadav, 2007). Past research efforts were found mainly focused on survey and disease indexing leaving inadequate attention to its long term management. It has resulted to massive decline of citrus orchards especially located below 1000 m altitude where population of vector – psyllid is abundant. Therefore, successful HLB management strategies particularly those of Brazil and Florida can be adopted in Nepal to save country's citriculture.

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